Boolean Network Models of the Fission Yeast Cell Cycle and Apoptosis

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Boole'sche Netzwerk-Modelle vom Zyklus der Hefezellteilung und der Apoptose

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ZUSAMMENFASSUNG

Gen- und Proteine-netzwerke beeinflussen alle Zellfunktionen und sind in ihren Eigenschaften sehr komplex. Die Vorhersage der dynamischen Prozesse innerhalb dieser Netzwerke sind daher zentraler Bestandteil der Systembiologie. Obwohl heutzutage auf zellularer Ebene im Bereich von Organismen Modelle von genetischer und molekularer Interaktion in weiter Ferne erscheinen, konnten aussagekräftige Modelle einfacher Signalpfade und kleiner modularer Molekül-Netzwerke an lebenden Zellen mit großem Erfolg untersucht werden. Diese bilden nun ein aktives Gebiet derzeitiger Forschung.

Um die Vorhersage der dynamischen Prozesse dieser Netzwerke zu ermöglichen, wurden verschiedene empirische und mathematische Methoden entwickelt. Die größte Herausforderung der mathematischen Ansätze besteht darin, die beeinflussenden Parameter der Netzwerke in Abhängigkeit von der Zeit darzustellen. Moderne Differentialgleichungsmodelle leisten dies, setzen jedoch die sehr genaue Kenntnis von Konzentrationen und der kinetischen Konstanten voraus.

In dieser Arbeit soll gezeigt werden, dass es möglich ist mittels Boolescher Netzwerke, die die zeitliche Aktivitätsfolge von Regulationsnetzen vorhersagen, aussagekräftige Modelle zu erhalten. Im Einzelnen heißt dies, dass die Kenntnis der kinetischen Parameter oder der exakten Konzentrationen nicht notwendig ist. Dies wird am Beispiel der allgemeinen Apoptose von menschlichen Zellen und dem Zellzyklus der Hefe (Schizosaccharomyces Pombe) gezeigt.

Ein Boolesches Modell der Apoptose wurde auf Basis biochemischer Datenbanken

entwickelt. Die dynamischen Eigenschaften des Apoptose-Modells lassen Rückschlüsse zu, dass es sich hierbei um einen robusten Prozess handelt. Dies begründet sich durch einen sehr dominanten Attraktor, welcher in direktem Zusammenhang zu dem auftretenden Zelltod steht. Der Einfluss wichtiger Proteine auf die Apoptoserate wurde in diesem Zusammenhang ebenfalls getestet.

Das zweite Modell, ein Boolesches Modell der Regulation von Hefezellteilung, basiert grundsätzlich auf bekannten biochemischen Reaktionsabläufen. Mittels dieses Modells ist es möglich, die Wildtyp - Sequenz während der Hauptereignisse der Zellentwicklung zu reproduzieren. Die dynamischen Eigenschaften dieses Modells zeigen, dass das Wildtyp Zellnetzwerk über einen dominanten Attraktor innerhalb seines Zustandsraums verfügt, welcher dem biologisch unveränderlichen Zustand des G1 entspricht.

Dieses Modell wurde mittels der Reaktion auf Störungen wie zum Beispiel Mutationen getestet. Durch die Reproduktion von Mutationen erhält man Ergebnisse über die Zuverlässigkeit des Modells, deren Schlüsselproteine und ein tiefergehendes Verständnis über die Kontrollmechanismen des Zellzyklus. Testverfahren wie diese zeigen, dass das Boolesche Netzwerkmodell eine große Anzahl von einfachen, doppelten und dreifachen "loss-of-function" und "overexpressed" Mutationen korrekt beschreibt.

Im letzten Teil dieser Arbeit betrachten wir zwei Verfahren, Differentialgleichungen und Boolesche Netzwerke, in ihrer jeweiligen Abhängigkeit voneinander am selben Beispielsystem, der Hefe-Zellteilung. Es konnte nachgewiesen werden, dass sich ein Boolesches Netzwerkmodell durch einen mathematisch wohldefinierten diskreten Limes eines Differentialgleichungsmodells herleiten lässt. Dies bildet die mathematische Grundlage, auf der sich Boolesche Netzwerke kontrolliert für die biologischen Regulationsnetzwerke anwenden lassen.

Die von uns innerhalb dieser Arbeit erzielten Ergebnisse begründen die Idee, dass

die Hefe-Zellteilung bis hin zu einem gewissen Grad als diskret bezeichnet und unabhängig von einem Zeitfaktor betrachtet werden kann. Häufig sind hierfür qualitative Details der biologischen Regulation ausreichend, um den Kontrollmechanismus von biologischen Prozessen darzustellen.

ABSTRACT

Gene and protein regulatory networks guide all functions in cells and are very complex. Most mathematical approaches for predicting the evolution over time of these networks have a common challenge – a demand of detailed information about the system, that is for example knowledge of exact concentrations and kinetic constants for the differential equation approach. In this thesis we show that Boolean models are able to reproduce sequential patterns of protein states with no demand on kinetic constants and exact concentrations. We demonstrate this on an example of a general model of apoptosis for human cells and of cell cycle of the simple eukaryote fission yeast (*Schizosaccharomyces Pombe*).

A general model of apoptosis is constructed on available data from biochemical databases. The dynamical properties of the obtained model indicate that apoptosis is a sufficiently robust process, since the system starting from different initial states reaches a fixed point that corresponds to the death of the cell. The model is verified via deleting a number of important proteins and observing the changes in apoptosis rate. The obtained results qualitatively reproduce observations in experiments.

The second model, Boolean model of fission yeast cell cycle, is also based merely on known biochemical reactions. The model is able to reproduce the wild-type sequence of events during main cell evolution phases. The dynamical properties of the model indicate that the wild-type cell network has a dominant attractor in state space that coincides with the biological stationary state, called G1.

The consistence of the model is tested on its response to different damages such

as mutations. The tests indicate that the Boolean network model captures a large number of single, double, triple loss-of-function and overexpressed mutations.

In the last part of this thesis we set two approaches – differential equations and Boolean networks in relation to each other with the same example system, the fission yeast cell cycle. We found that the Boolean network can be formulated as a specific coarse-grained limit of the more detailed differential network model for this system. This lays the mathematical foundation on which Boolean networks can be applied to biological regulatory networks in a controlled way. The limitations of the Boolean approach are also discussed.

The results of this thesis support the idea that the nature of the fission yeast cell cycle is discrete to some certain degree and that the timing is not always a crucial factor. Therefore, qualitative data may be sufficient to grasp certain parts of control mechanisms of biological processes.

CONTENTS

1. INTRODUCTION

Predicting the dynamics of cellular biological processes that control living organisms is a central challenge of systems biology. These processes are guided by sophisticated networks of interactions between macromolecules of the cell such as proteins, nuclei acids and polysaccharide. Their complexes and structures define the unique properties that enable them to perform the functions of the cell as for example, catalysis of chemical transformations, production of movement and heredity.

Nowadays, we have a lot of knowledge about individual cellular components and their functions. However, these individual properties cannot entirely describe any biological function, since it is very rarely that a single molecule is the only molecule that participates in a biological process. Instead, most biological characteristics arise from complex interactions between a cell's components. Therefore, it is necessary to understand the structure and dynamics of the complex intercellular web of interactions that contribute to the structure and functions of a living cell.

The development of experimental techniques, such as microarray analysis provides the data of the status of a cell's components at any given time. Other experimental technique – chromatin-immuno precipitation ("chIp") experiments or Yeast Two-hybrid screens, help to determine how and when these molecules interact with each other. A big variety of networks emerge from the sum of these interactions, such as protein-protein interaction, metabolic, signaling, and transcription-regulatory networks. All these networks are dependent and are responsible for the behavior of the

cell. The task of system biology is to integrate theoretical and experimental techniques for building predictive models.

If the biochemical details of a chemical molecular network are known, standard techniques are at hand for their computer simulation. A method capturing molecular details is chemical Monte-Carlo simulations [65, 66]. Less computationally costly and perhaps the most commonly used approach to modeling biochemical pathways and networks are differential equations which capture the underlying reaction kinetics in terms of rates and concentrations [69]. This method is highly developed today and is broadly applied to predictive dynamical modeling from single pathways to complex biochemical networks [206].

Such mathematical models contain detailed information about the time evolution of the system which, in some circumstances, is more than we are interested in. For many biological questions, knowledge of the sequential pattern of states of the central control circuit of a cell would be a sufficient answer, as, for example, in cell cycle progression, cell commitment (e.g. to apoptosis), and in stem cell control and differentiation. When we are interested in the path that a cell takes, the exact time course of the control circuit dynamics may not be needed, however, its modeling takes most effort and often one needs to know large numbers of biochemical parameters that are not easily obtained [191, 204].

Indeed, recent research indicates that some molecular control networks are so robustly designed that timing is not a critical factor [25]. Vice versa, as a working hypothesis, this observation bears the chance for vastly simplified dynamical models for molecular networks, as soon as one drops the requirement for accurate reproduction of timing by the model, just asking for the sequence of dynamical patterns of the network. Recent studies demonstrate, that such more simplified models indeed can reproduce the sequence of states in biological systems. For example, a class of discrete dynamical systems with binary states, mathematically similar to models used

in artificial neural networks, has recently proven to predict specific sequence patterns of expressed genes as observed in living cells [7, 123].

Such models are in the mathematical tradition of random Boolean networks which, for decades, served as a simplistic analogy for how gene regulation networks could in principle work [105]. In these historical studies, dynamical properties of random networks of discrete dynamical elements were studied to derive possible properties of (the then hardly known) regulatory circuits [105]. In the new approach outlined above, however, similar mathematical elements now serve to simulate one specific known biological control network. From a different perspective, they can be viewed as a further simplification of the differential equation approach [19]. Recent application of this model class to modeling real biological genetic circuits show that they can predict expression pattern sequences with much less input (e.g. parameters) to the model as the classical differential equations approach. Examples are models of the genetic network underlying flower development in *A. thaliana* [55, 145, 197], the cell-cycle networks of *S. cerevisiae* [123], signal transduction network for abscisic acid induced stomatal closure [124] and of the mammalian cell-cycle [56], as well as the segment polarity gene network in *D. melanogaster* [7, 173].

For example, the model by Albert and Othmer [7] of the segment polarity gene network in *D. melanogaster*, as well as the model by Li et al. [123] of the *S. cerevisiae* cell cycle control network, yield accurate predictions of sequential expression patterns, previously not obtained from such a simple model class. In these models, the dynamics can be viewed in terms of flow in state space of possible states of the network, converging towards so-called attractors, or fixed points, which here correspond to specific biological states. These attractors and their basins of attraction in state space mainly depend on the circuitry of the network, and their analysis yields further information about the robustness of the dynamics against errors or mutations.

How generic is this approach? Here we address the question whether the approach

of discrete dynamical network models is a more general method, namely whether constructing predictive dynamical models for gene regulation from Boolean networks is a straightforward procedure that generalizes to other organisms.

In this thesis we build two Boolean models. First, we construct a general model of apoptosis for a human cell (chapter 4). Apoptosis, a cell suicide, is a vital process during development, differentiation, proliferation, and cell termination. The lack of apoptosis is associated with cancer. This process is very complicated and involves a large number of proteins. Due to the absence of detailed information on biochemical reactions, previous attempts to model this process with differential equations ended up with simulating only some small parts of the pathways. The dearth of integrative model and very limited data on reaction rates, motivated us to use the Boolean networks approach for constructing a general apoptosis model for a human cell.

Secondly, a Boolean network model is constructed for well studied process, the fission yeast cell cycle. We choose the fission yeast (*Schizosaccharomyces Pombe*) cell cycle as an example system that on the one hand is well understood in terms of conventional differential equation models, but on the other hand is markedly different from the above examples, as *S. cerevisiae*. *S. Pombe* has been sequenced in 1999 and has been used as a model organism only relatively recently [60]. Models exist [153, 154] that mathematically model the fission yeast cell cycle with a common ODE (ordinary differential equation) approach. These are based on a set of differential equations for the biochemical concentrations that take part in the network and their change in time (and space). This approach allows to predict the dynamics of the fission yeast cell cycle for the wild-type and some known mutant cells [204, 205].

We will in the following construct a discrete dynamical model for the fission yeast cell cycle network. An interesting question will be, how far we will get without considering parameters, as kinetic constants etc., that are a key ingredient of the existing models. We will base our model on the circuitry of the known biochemical

network, only. Chapters 5 and 6 describe the Boolean network model for wild-type fission yeast cell cycle. In chapter 7 the extended version of the Boolean network model is represented which captures a large number of mutations.

Further, in chapter 8 we put another question, how this Boolean network method is related to other methods, in particular, how the Boolean network approach is related to differential equation approach. The two diverse methods are both based on the same "wiring" diagram of interactions between the components, however, use much different amounts of information about these interactions. We further explore the correspondence between ODE and Boolean network models considering a specific biological system and demonstrate how a working Boolean model can be derived in terms of a mathematically well defined coarse-grained limit of an underlying ODE model. As our working example we choose the same process – the fission yeast cell-cycle control network (*Schizosaccharomyces Pombe*). All results are summarized in chapter 9.

2. BIOLOGICAL PRINCIPLES OF GENE REGULATION

Networks of biochemical interactions guide all biological processes in living organisms. Even within an individual cell these networks of interactions display an incredible complexity. In this chapter we introduce the basic biological principles underlying regulatory mechanisms that are needed to comprehend the results of this thesis. The results from recent biological research with implications on this will be covered, as well.

2.1 Regulatory networks

In order to understand the mechanisms of biological processes on the molecular level, one has to comprehend the regulatory systems that control them. All cellular processes, such as differentiation, replication, response to external environmental signals are guided by regulatory networks [100]. The regulatory networks are composed of interactions between a collection of DNA segments, RNA, proteins, nuclei acids, and polysaccharides. The central role in controlling the interactions is played by the genome. The genome is the full set of genes of an organism, contained in the chromosomes. Although all cells in an organism, comprise the same DNA, they are very different. The reason for this is that different DNA segments (genes) are expressed under control of regulatory mechanisms and various environmental signals. Different gene expression triggers synthesis of different proteins, which induces different cell functions.

The proteins perform all kinds of biological processes. They have very various functions – for example, they can work as transcription factors, enzyme catalyzing metabolic reactions, components signaling transduction pathways or building blocks. The function of every protein is determined by its structure. Every protein has its own unique amino acid sequence which is determined by the nucleotide sequence of encoding protein gene. For protein synthesis the genes, encoded in DNA, first are transcripted into pre-mRNA. It is followed by post-transcriptional modification to form the mature mRNA, which is then used as a template for protein synthesis. As the mRNA has been transported into the ribosome, the translation process takes place resulting in protein synthesis. The process of protein synthesis is controlled by other proteins at every step: RNA processing and transcription, RNA translation and post-translation modifications of proteins, i.e. for example, transcriptional factors can bind to regulatory sites of genes and may initiate, enhance or inhibit the transcription of a gene. Some proteins can perform or change their functions if they bind to other proteins and form a complex. The cascade of interactions between different cellular components can be triggered by an internal or an external signal [8].

This all contributes to complex regulatory mechanisms formed by networks of interactions between DNA, RNA, proteins, nuclei acids and polysacharydes (see Fig. 2.1). Let us give a simple example of a gene regulatory network. In Fig. 2.1 gene a is repressed by proteins B and C. Proteins A and D form a heterodimer and bind to gene b, thereby negatively regulating it. Binding of repressor proteins prohibit gene transcription [100].

The intensive study of regulatory networks was triggered by new experimental techniques such as cDNA microarrays, oligonucleotide chips, mass spectrometric identification, serial analyses of gene expression, two dimensional electrophoresis with mass-spectrometric identification [27, 101, 127, 128, 135, 158, 235]. The available databases, such as the KEGG database, contain information on the function and

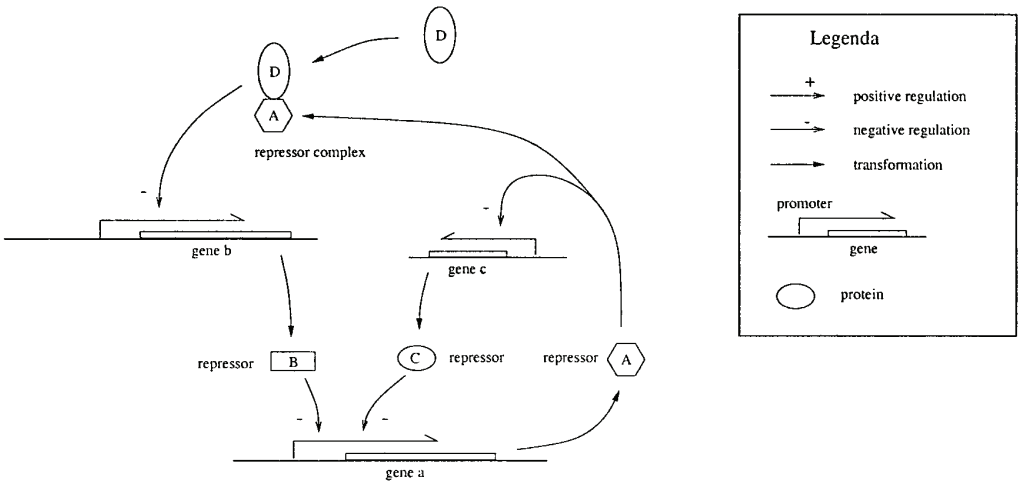

Fig. 2.1: Example of a generic regulatory system, consisting of a network of three genes a, b and c, repressor proteins A, B, C and D and their mutual interaction. Image is taken from [100]

structure of approx. 110,00 genes for 29 species [104] and are also used for comprehending the regulatory mechanisms. These experimental techniques and databases provide an incredible quantity of expression and interactions data. The most challenging task is to integrate this knowledge into a system-level understanding and thereby reveal the regulatory mechanisms that provide the emergence of complex patterns of behavior from available experimental data.

The complexity of regulatory networks is so high that an intuitive understanding of controlling mechanisms is hardly possible. Therefore, mathematical and computer models have to be applied for this purpose. The requests for mathematical models of cell biology were pin-pointed by Hartwell, Hopfield, Leibler and Murray: " The best test of our understanding of cells will be to make quantitative predictions about their behavior and test them. This will require detailed simulations of the biochemical processes taking place within [cells]. We need to develop simplifying higher-level models and find general principles that will allow us to grasp and manipulate the functions " [206]. The next section gives a description of existing mathematical

approaches and discuss its appropriate use for cell biology.

3. MODELING REGULATORY NETWORKS

This chapter gives an overview of existing mathematical approaches that are used for modeling regulatory networks. We pay additional emphasis on differential equation and Boolean network modeling approaches as methods employed further in this work and set up in correspondence in chapter 8. The special attention is paid to the question on why the Boolean network approach is appropriate for modeling regulatory networks.

3.1 Modeling Regulatory Networks with Differential Equations

The development of new experimental techniques mentioned in the previous chapter, has created a strong need for working out new mathematical models that allow to understand the obtained experimental results. The first step in constructing all kinds of mathematical models of regulatory networks is to assemble the components and the interactions between them. The next step is to complement this with information or hypotheses concerning the dynamics of interactions, since while the biological process proceeds, the expression of genes and activation of proteins can constantly change [3]. Therefore, the models simulating regulatory network should have a dynamical aspect, i.e. they need to have a set of components with defined state for each of them and investigate how these states change by the interactions in the network. The state can, for example, correspond to concentration of a protein or have a binary value denoting that a protein active is or not.

A complete gene or protein regulatory network model includes knowledge on a set of participating components and interactions between them as well as a set of initial states of these components. The model should be capable to lead to the known final state or a set of states and correctly perform the dynamical behavior of the regulatory network. The motivation for the modeling is that the validated models can explore the cases that are difficult or impossible to investigate experimentally and moreover to make predictions and to gain insights on regulatory mechanisms.

Definitely every model is an abstraction of reality, since the modeling of all details of biological processes are in most cases impossible due to the incompleteness of experimental data and computational limits in numerical simulations. Every modeling approach includes its own set of assumptions that are made *a priori*. Therefore, one is looking for the simplified descriptions that neglect details of biochemical reactions but capture the essential properties of the process. This approach is typical in physics, but may be rather tricky in molecular biology. The reason for this is that biological processes take place at different scales, both in time as well as in space, and it is often not easy to decide which level of abstraction should be chosen to describe the biological process correctly. It is necessary to understand which abstractions are possible and which details are crucial for a process. Every researcher faces this challenge of choosing an approach and often it leads to the question: Whether to model a process with a smaller number of components and more details for every particular component, or to include a larger number of components but less details for each of them. Which way one should choose to comprehend the behavior of the system depends on the particular biological system. Generally, all models can be separated along two characteristics: discrete or continuous and deterministic or stochastic [4]. In this chapter we give an overview mostly of deterministic mathematical methods for modeling regulatory networks and show biological processes for which they were applied. The section is accompanied by a comparative analysis of these methods. Among all

methods we put two into focus – differential equations as a most widespread method and Boolean networks, as the approach that is used in the current thesis as a main method for modeling. In chapter 8 we put these two methods into correspondence to each other.

3.1.1 Nonlinear Differential Equations

The most widespread approach to modeling biochemical networks is via differential equations, based on the known chemical kinetics and successfully applied to describing numerous processes in living organisms [34, 151, 153, 154, 155, 205]. To build an ODE model, one starts with a schematic diagram representing the known interactions between components. Then this diagram is converted into a set of differential and algebraic equations using the principles of biochemical kinetics. In many cases the reactions are described by mass action, Michaelis-Menten or Goldbeter-Koshland functions, when the transition time is very short [100, 117, 206]. The full ODE model then consists of this set of rate equations, a set of parameter values plus a set of initial conditions. The solutions of the ODEs give the time-dependence of each component of the system. In practice these solutions depend on rather detailed knowledge about all reactions and kinetic parameters. The general form of these equations is the following

$$\frac{dx_i}{dt} = F_i(x), 1 \leq i \leq n \tag{3.1}$$

where $x = [x_1, ...x_n] \leq 0$ is the vector of concentrations of participating components, and F_i is the rate of concentration change of x_i. F_i is usually nonlinear Hill-like function having a sigmoidal shape.

If it is necessary to take into account time which is required to complete different biological processes such as transcription, translation, the equations can be

represented with differential time-delay equations

$$\frac{dx_i}{dt} = F_i(x_1(t-\tau_{i1}), ..., x_n(t-\tau_{in})), 1 \leq i \leq n, \tag{3.2}$$

where $\tau_{i1}, ...\tau_{in} > 0$ are discrete time delays.

Due to the non-linearity of F_i it is in most cases difficult to find an analytical solution for a set of equations such as (3.1) or (3.2), therefore, such equations are usually solved numerically.

Thus, having an ODE model for a particular process at hand, it is possible to predict the systems behavior under various conditions, by changing the necessary constants or initial conditions and running the modified model. On the other hand, this would provide data without any insights on the underlying mechanism. For understanding the mechanism, the tools that comprehend why a system performs a specific behavior and how it is connected with parameter values are needed.

For this purpose, bifurcation analysis is applied, which allows to determine how the system's dynamics change as a function of different parameters, in particular to characterize the types of solutions one can expect to find for a system of ODE [206]. The most interesting are is "recurrent" solutions: steady states, when concentrations do not change in time, and oscillatory solutions, when the variables go through the same sequence of states periodically. The properties of recurrent solutions depend on the exact values of the parameters of ODE and experience a qualitative change in a bifurcation point when a smooth change of parameter's values causes a sudden qualitative change in the dynamical behavior of the system.

The behavior of the system can be explored by plotting a one-parameter bifurcation diagram. To build a bifurcation diagram, some physiologically relevant quantity, the signal, is chosen as a bifurcation parameter, which causes the changes in behavior, in the "response" of the regulatory system. Scanning different values of the signal,

the steady-state values of the signal are plotted as a function of other variables. At the bifurcation points the behavior of recurrent solution changes rapidly. The application of bifurcation analysis in a number of models [151, 154] helped to find different behaviors which correspond to well-known physiological states of the cell as well as states that had never been recognized experimentally. The application of bifurcation analysis is shown in chapter 6 on the example of an ODE model for the fission yeast cell cycle.

Also some special investigations were made to reveal the relationship between feedback loops and the resulting dynamics in a system [36, 75, 77, 78, 108, 203]. It has been shown that in case of a negative feedback loop the system has a single steady state or oscillates, whereas the positive feedback loop tends to settle the system in one of two stable states, depending on the initial conditions. For more detailed information, one can read the paper [206], which gives a review on different topology of positive and negative loops that generate different dynamical behavior with examples of related biological systems.

The ODE approach was successfully used for modeling a large number of biological processes, as, for example, 11-node differential equations model of signaling network for programmed cell death after infection of *Arabiodopsis thaliana*, which allowed to refine the signaling circuitry. The other most known examples are genetic regulatory process for induction of the lac operon in *E.coli* [18, 29, 134], the development cycle of bacteriophage T7 [54], the synthesis of *trp* in E.coli [114, 162], the expression of a human immunodeficiency virus (HIV) [83], and circadian rhythms in Drosophila, budding yeast, fission yeast and mammalian cell cycle [201, 30, 233], as well as other organisms [119, 170] .

The main difficulty one meets working with ODE is the lack of kinetic parameters in the rate equations. The kinetic rates are often out of reach of current experimental techniques. Modern reverse-engineering techniques are limited in the amount

of information they are able to extract from current databases. Therefore the typical procedure is adjusting kinetic parameters through numerous simulations of the model and comparing the results with available experimental data. At this point one has to mention that deriving large differential equation system is a challenge and this problem becomes almost not-solvable in case of large differential equation system. Therefore, the number of participating components is limited due to technical problems in finding the right parameters for large systems.

- $Piecewise-linear\ differential\ equations\ (PLDE)$

One option to work around the difficulties of the ODE approach is to use simplified approximated models such as piecewise-linear differential equations. The main simplification of PLDE is that behavior of gene which was previously regulated by sigmoidal curves, such as the Hill function, is substituted by a discontinuous step functions, thereby the certain activity of gene or protein can be triggered only when concentration of its regulating protein/gene is above the defined threshold [100]. This rule is motivated by the switch-like behavior of many interactions in regulatory networks (the foundations of switch-like behavior of regulatory networks will be discussed in detail in section 3.3). The resulting differential equations are piecewise-linear and are related to the logical models.

Although the global behavior of the PLDEs is still complicated and not understood in general, compared to ODE the mathematical analysis of PLDSs is simpler because of the form of equations, and finding dynamical properties such as steady states is easier. PLDSs can be also analyzed qualitatively by discretizing. Moreover, the research results of Glass and Kauffmann [68, 69, 70] suggest that there is no difference in the qualitative properties of the solutions of ODE and related PLDE, therefore step functions instead of Hill function can be used. In chapter 8 for the example of the fission yeast cell cycle model we will show that even a transformation of an ODE model into a totally discrete system keeps the essential dynamical properties of the system.

PLDE models have been employed for investigating the dynamics of the regulation of trytophan synthesis, arabinose catabolism and nutritional stress in E.coli, initiation of sporulation in Bacillus subtilis, as well as quorum sensing in Pseudomonas.

Systems of piecewise-linear differential equations can also be analyzed qualitatively by discretizing and transforming them to qualitative differential equations. In this type of equations, functions F_i are reduced into sets of qualitative constraints and variables and their derivatives take qualitative (discrete) values [100, 3].

Coming back to our question which method is appropriate for this or that process, we would like to note that even thought differential equations give a very detailed description of a biological process, in some processes this detailed time information is more than what we are interested in. Some recent studies show that essential characteristics of the system are rather robust to variations in parameter values, i.e. kinetic constants and concentrations. For example, for a segment polarity network in Drosophila has been shown that the essential properties of the system appear to be robust and even are not influenced by large variations in parameter values [216]. Another example is a model of bacterial chemotaxis [11], for which it has been suggested that the network topology rather than exact values of parameters determine the robustness of the system. Therefore for such systems more simple models can be appropriate, that are able to reproduce the right sequence of activation patterns of proteins/genes without exact timing, such as Boolean networks which are described in section 3.2.

3.1.2 Other types of differential equations used for modeling regulatory networks

In the previous section it was assumed that 1) regulatory systems are homogenous and 2) the discrete nature of molecular components and the stochastic character of their interactions can be neglected. These assumptions are not always appropriate, in particular, the homogenous assumption is not valid in the case of embryo development

for which it is necessary to take into account gradients of protein concentrations across cell tissues. The second assumption that regulatory networks can be modeled with continuous deterministic models are valid for reasonably high molecular concentrations, whereas for low concentrations the discrete interactions become very important which have stochastic nature.

This subsection comprises only a brief overview of modeling approaches suitable for these more complicated cases.

• *Partial differential equations* (PDE)

Biological processes are the complex set of events coordinated in time and space. The differential equations, described above, neglect the spatial dimensions, therefore the gene and protein regulatory networks are assumed to be spatially homogenous. However, these assumptions are not always appropriate and there are processes for which it is necessarily to take into account where in a cell the particular process takes place. For instance the development of an embryo into an adult organism involves spatio-temporal differentiation of around 10^{10} cells. In these cases partial differential equations is an appropriate method to use [100].

This approach has been used for research in embryo development, for example, for investigation of the emergence of segmentation patterns in the early Drosophila embryo [94, 118, 141, 142, 143]. There are typically two main difficulties of this approach – lack of data and dimensionality. The first difficulty is due to the fact that measurements of absolute expression levels are difficult to obtain [53]. The dimensionality difficulty refers to the fact that the models have to be simple enough to simulate it within a reasonable period of time. Moreover, the predictions of PDE models are sensitive to the shape of the spacial domain and the boundary conditions. Therefore, having a big potential in detailed representation of a biological process, due to the mentioned difficulties, PDE models are usually strong abstractions of biological processes [53], since for complex models the task of finding the right parameters

reproducing the experimental data is extremely difficult.

- *Stochastic master equations*

All approaches described above neglect the discrete and stochastic nature of interactions between the molecules. The evolution of components is not deterministic and the discrete models that allow uncertainty and noise better capture the real dynamics of biological processes. Consideration of the stochasticity becomes especially important in case of low concentrations [64, 66, 113, 133, 147, 165, 193] on a molecular level, due to the fluctuation in timing in different cellular events such as transcription. This may result in that even thought two regulatory networks have the same initial conditions, they may reach different states. Therefore, many authors [12, 132, 147, 165] propose to use discrete and stochastic models of gene regulation – stochastic master equations.

Such models provide a very detail picture of biological processes, abut on the reverse side one has to pay for it: Master equations are even more difficult to solve analytically and numerical simulations are much more complicated, as well. To make it easier, master equations can be approximated by stochastic differential equations [147, 210], or directly simulated by the Gillespie algorithm.

A stochastic master equation model has the highest potential to describe the real system in the greatest detail. On the other hand it requires even more detailed knowledge on reaction mechanisms. Whether these costs make sense or not depends on the level of granularity at which the investigator aims to study the biological process. On a larger time-scale, stochastic effects often do not play a major role, so that deterministic models can be a good approximation [126].

The next section is devoted to Boolean network models.

3.2 Boolean networks

3.2.1 Discrete-state approach

The limitations of available data (thermodynamic constants, rate constants, concentrations), difficulties in finding analytical solutions and simulating large-component models, have motivated alternative simplified modeling methods. The discrete state approach is based on an assumption that every component of a regulatory network has a small number of discrete states and the interactions between them are described by logical functions. Several discrete modeling methods have been worked out: Boolean networks, generalized logical networks, that allow to have more than two states to each component and use asynchronous update, rule-based formalism, Petri networks. We concentrate here on a Boolean networks modeling approach, which is used as the main modeling method of the current work.

3.2.2 Boolean networks. Definitions

The central idea of Boolean networks modeling is that a gene or protein is treated as a discrete, binary element that has only states ON/OFF. An assembly of such elements forms a network of interactions. The ON state of the element can have a rather general meaning. Genes in the ON state can denote 'transcribed', whereas for proteins it can label 'in active conformation' , e.g. phosphorylated/unphosphorylated or high concentration.

All types of interactions are reduced to activation/inhibition and described by logical functions. For example, if for activation of some protein two other proteins should be active, a Boolean function 'AND' can be used, whereas if the activation of only one from these two proteins is necessary, a Boolean function 'OR' would be appropriate.

Let us further introduce the terminology used in Boolean networks modeling approach. Each element (protein/gene) in Boolean network of a node in the network that can be in two states 1 or 0 ($S_i = 0; 1$), corresponding to the ON or OFF state of the element, respectively. A network of n elements S_i ($i = 1, 2, ..n$) at any given discrete time t can be represented as a state vector

$$S(t) = (S_1(t)...S_n(t)). \tag{3.3}$$

The entirety of all network states forms the n-dimentional *state space* – the system has 2^n possible states.

The interactions between the elements are the edges of the network. The state of the node in the next time step is determined by Boolean (logical) function, that defines how the incoming connections (inputs) modify the state of the (respective) receiving node (output). For example, if node i has k connections to the other nodes, then the state of node in the next time step is determined by the states of these k nodes at the previous time step:

$$S_i(t + 1) = f_i(S_1(t)...S_k(t)). \tag{3.4}$$

Thereby, a Boolean network is defined by a set of nodes $v = x_1...x_n$ and a list of Boolean functions $F = f_1...f_n$. The model usually is represented in the form of a directed graph. All nodes are updated synchronously. Since the dynamics is deterministic and the majority of states are 'logically unstable', execution of the Boolean rules (network updating) drives the network state along distinct *trajectories* through "transient states" until they attain states that will transit onto themselves upon executing the Boolean function. Such states are the *attractor* (states) of the network. An attractor can also consist of a small (relative to n) set of states that transit into each other in a circular manner (limit cycle attractors) or in fixed-point

attractors. All those states in state space that fall into the same attraction of the attractor form the basin of attractor.

A particular characteristics of attractor states is robustness. If attractor state is robust, a small perturbation (random flipping of the ON-OFF states) of individual nodes of the networks will lead to transient states that may move back to the previous attractor states. In non-robust case the system will end up in some other attractor.

3.2.3 Random Boolean network model (RBN)

The first Boolean network model applying to biology was introduced about 40 years ago by S.A. Kauffman. At that time the available experimental data was very limited and Kauffman represented regulatory genetic networks as random Boolean networks (RBN). Although, first models did not represent specific biological regulatory networks, nevertheless they raised a lot of discussion in the scientific community and played a vital role in further development of Boolean network research. Moreover, RBN theory established terminology and analyzing tools that are used nowadays in modern non-random Boolean networks. Therefore, it is necessary to demonstrate the results of RBN not only as a part of history of research, but also because RBNs can be used as a "null' model, which is a reference for the modern Boolean networks. In particular, in chapter 4 and 6 we compare our Boolean models of fission yeast cell cycle and apoptosis with a corresponding RBN networks to find out whether the real biological networks share the properties of RBN or they evolved in a such way that they have its own very special dynamical characteristics.

In Kauffman's pioneering work [105] it was assumed that genes are equivalent and can be represented as nodes. Every gene receives inputs from a fixed number (K) of randomly chosen genes (nodes) of the network [5, 105]. The whole network is a set of N genes (N nodes). The dynamic behavior, i.e. whether a certain gene at the next moment will be ON or OFF is in accordance with Boolean (or logical) function of

update. The most widespread Boolean functions are canalizing and threshold type functions. A canalizing function is a Boolean function with a property that one of its inputs alone can determine the output value. A threshold function is a Boolean function whose input depends on the sum of its inputs, only. This is motivated by the finding that gene regulatory functions are biologically meaningful [84, 107]. In the current work both types of update function were used – the first one for an apoptosis Boolean network, the second one for the fission yeast cell cycle. In RBNs the logical functions are randomly selected from the 2^{2^k} possible k-input Boolean functions for every node and then kept fixed. All nodes are updated synchronously [62].

In RBNs, the critical parameter is the connectivity (K), according to which networks can be classified into three regimes: frozen ($k < 2$), chaotic ($k = 2$) and critical ($k = 2$). Every regime has typical dynamical characteristics.

A common test for the dynamical property is the "spreading of perturbations" or "robustness to perturbations." We can "mutate" a node of an RBN by flipping its state and measure how much a random damage affects the rest of the network, in particular, compare evolution of the original and perturbed network. In the frozen phase the perturbed network returns to the same path of the original network. At the critical regime perturbation can spread through the network and affect the states of nodes, but not necessarily all the network. In chaotic phase, the perturbation propagates through the whole network.

Another feature is the "sensitivity to initial conditions". In the frozen phase, similar states tend to converge to the same state, at the critical regime they tend to stay on trajectories that neither converge nor diverge in state space and in the chaotic regime they diverge.

The dynamical properties of every regime are characterized by typical cycle lengths and number of attractors. Kauffman concentrated his attention on critical networks (k=2) and basing on results of computer simulations for the networks sizes reachable

at that time, obtained that the mean number of attractors and the mean length of attractors grows as $\sqrt{N}$. Kauffman associated attractors with cell types, since every cell has the same DNA, different cells are distinguishable only by the pattern of gene activity. The results of Kauffman looked realistic at that time, since data at late 60's indicated that the number of cell types is proportional to the square root of the number of genes. It was also assumed that the mass of DNA is proportional to the number of genes. Therefore it seemed at that time that complex reality can be described with a simple model of RBN [47].

However, modern biological data refute it: Number of genes appear to be proportional not the the mass of DNA but much smaller for higher organisms. Moreover, calculations of RBN with modern powerful computers have shown that for large N the attractor number and length grows faster than any power law.

Later it has been found that molecular and genetic networks have scale-free topology, which triggered a development of modified RBN networks that have not a constantly fixed connectivity, but a scale-free k-distribution. The investigations showed that the networks properties at each regime are similar to homogeneous RBNs. It was also demonstrated that evolability is easier in scale-free networks, since that can adapt even in frozen regime. For solving a typical problem of incomplete genomic data, Smulevich proposed probabilistic Boolean networks, which are useful for inferring possible gene functionality from existing data [178].

A large input into Boolean networks in biology was made by Rene Thomas. He proposed logical rules for different mechanisms of transcription regulation and included multilevel variables in the network [198, 199]. Later Thomas together with Thieffry focused their research on a role of feedback loops in regulatory networks [196]. The loops can be divided into two groups: If the number of inhibitory interactions in the loop is even/odd, the loop is positive/negative respectively. Positive loop is a necessary condition for existence of multiple states in a system, whereas negative

feedback loops guarantee stable limit cycles. They were the first who proposed to separate different time scales and asynchronous update taking into account that genes do not match in step, thereby escaping from the main criticism of classical RBN – the synchronous update of all nodes. Thomas and Thieffry also discussed when and how the variables with more than two ON (1)/OFF (0) values should be used [199]. Thieffry was a pioneer in global characterization of transcriptional regulation in Escherichia coli in terms of connectivity and topology [196] and first developed a predictive dynamical Boolean model for Drosophila Gap-Gene system [207].

Another variation of classical RBN models was introduced by Kauffman and Glass in 1973 [69] and later continued by Kappler in 2002 [102] in which genetic regulatory networks include continuous states. In these models the gene interactions are incorporated as logical functions using differential equations.

Thus, RBNs triggered the development of Boolean networks models for biological systems. RBNs can also be used for studying evolvability of regulatory networks at an abstract level [21, 25, 62]. However, the question arises whether the real regulatory networks share the same properties as RBN, i.e. how high is the potential of RBN for characterizing real systems. We attempt to answer this question by analyzing the Boolean networks for real processes – cell cycle and apoptosis and comparing them with corresponding RBN models in chapter 5,7, and 9.

The next section is devoted to the validation of Boolean network models, followed by an overview of recent Boolean networks models.

3.3 Why Boolean models are appropriate in biology

The abstraction of gene or protein activity to two states (ON/OFF) is justified with a threshold behavior and bistable switches which are widely observed in regulatory networks [206]. Threshold behavior has been found in many systems: MAPK signaling

pathway, heat experiment in *E.Coli* [22], fission yeast, budding yeast and mammalian cell cycle [206], different apoptosis pathways, etc. A rapid switch-like behavior has been shown by many proteins in most cases composed of multiple subunits [24], as for example the enzyme aspartate transcarbomaylase. It has been also revealed that particular biochemical reactions on surfaces and 'transcriptional complexes' are characterized with switch-like behavior [92].

The threshold behavior was directly observed in cell experiments at single cell resolution level [92](Fig. 3.1). In particular, a threshold behavior has been demonstrated between the amount of an inducer and the expression of dependent marker – a bimodal distribution with low and high expression of the marker has been observed. This argues that within a single cell the probability of transition between two discrete states even in the presence of noise has a gradual response. Moreover, stochastic fluctuations in the level of reactants, due to low concentration of mRNA will induce sensitivity amplification, i.e. promote a threshold behavior.

From a mathematical point of view a switch-like behavior and bistability occurs due to a steep sigmoidal input-output relation. Dynamics of many biological processes such as apoptosis, cell cycle of budding yeast, fission yeast and mammalian cell cycle is described by sigmoidal functions that give rise to characteristic threshold behavior and bistability [92].

Bistability in a simple case of two proteins, say A and B (in Fig. 3.2) results in that the system can have two stable states: Concentration of A is low and B is high and *vice versa*. In which of these two states the system stays is determined by initial condition.

In bigger systems this kind of 'sharpening behavior' can be realized with more sophisticated control, for example, cascades provide switch-like behavior between multiple distinct state, performing multistability [69].

The bistable behavior is often the result of positive feedback loops in biological

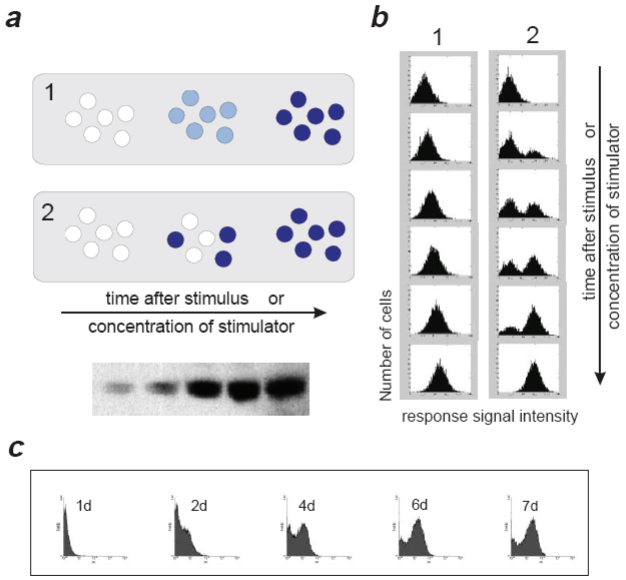

Fig. 3.1: All-or-nothing response for protein marker induction in individual cells. Two types of response (1) and (2) would give the same results in a biochemical assay, because of averaging over a cell population; The concentration of the stimulator (dose-response experiment) increases over time. In case (1) every cell gradually increases its expression of the induced marker protein. In case (2) increasing number of cell abruptly express the marker. The threshold for the all-or-nothing response in (2) shows a distribution (see *b*) because of noise. Therefore cells switch at different concentrations and times for higher expression level. b. Simulation of flow cytometry for the two responses (1) and (2). In case (1) the mean of the distribution gradually shifts with increasing of dose concentration. In case (2) the increase of dose leads to a bimodal distribution, where the ratio of the size of the two peaks changes, not their position. c. Experimental results for *b*. Here HL-60 cells are stimulated with retinoic acid to induce expression of CD 11b, measured by flow cytometry. The shifting and bimodality of the peak size over time indicate that individual cells exhibit a switch-like response to the inducer. The figure is taken from [92]

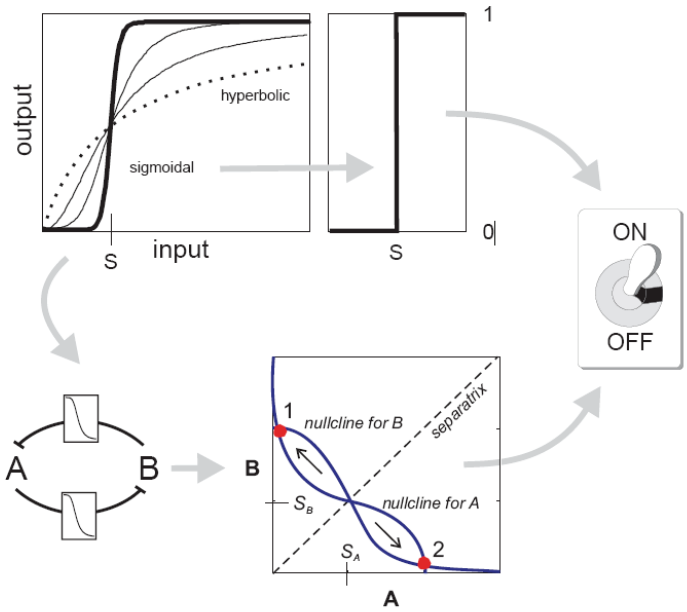

Fig. 3.2: Approximation of molecular interaction with switch-like function. a. Threshold behavior of a system (see text) leads to a steep sigmoidal input-output function with a threshold instead of the hyperbolic function (dotted line). This can be approximated with a step function. The output is OFF/ON if the input is below/above the threshold respectively. b. The sigmoidal function can lead to bistability; For example, if two gene products, A and B inhibit each other, whereby the rate equations for inhibition obey a sigmoidal function as shown in the small insets. On the right side the resulting behavior is represented. Axis denote the activity of A and B respectively. The state space is divided by a separatrix (dotted line). The solid curves are the nullclines for A and B, which intersect each other in dots 1 and 2 (stable states 1 and 2). If the initial conditions are above (below) the separatrix, the activity of A and B will be attracted to the state 1 (state 2) respectively. The figure is taken from [92]

regulatory networks. "Positive feedback is a set of regulatory steps that feeds the output signal back to the input" [23]. The increase in output activity raises the input signal, thereby amplifying the output signal. The biological examples of networks with positive feedback loops include Ca2+ spikes [140], chemotaxis [144], oocyte maturation [229]. These feedback loops are one of the most important regulatory motifs in cell signalling [10]. For such systems, if the input is below a critical threshold, the output remains near its basal state. For inputs above the threshold, the output increases to a high active state. The important feature of such systems is hysteresis. To remain in the active state, the input signal can be lower than the input signal required for triggering the initial transition from basal to active state.

Therefore, the state of a system depends on whether the initial concentration of a protein is below or above the corresponding threshold. In the Boolean network model, built in chapter 6, this characteristic is realized in the following way. The protein can be activated by the resulting incoming activating signals being above the threshold, whereas if their value exactly coincides with a value of a threshold, the protein keeps the previous state, i.e. if it was ON it stays ON and vice versa.

Thus, all-or-nothing behavior appears to be widespread in biological regulatory networks. Definitely, representing a gene or a protein as ON or OFF is an abstraction, since they may have multiple activity states. One way to solve this is to allow different discrete levels of activation for genes (nodes), which was realized in [145]. An alternative way, proposed in our work, is the representation of every node of multiple-state activity with a separate node, which has its own threshold of activation.

Therefore, discretization of protein or gene activity states appears to capture the true behavior observed in regulatory networks, whereas time discretization and synchronous updating do not have experimental validity and solely represent the coarse-graining. In Boolean network models it is usually assumed that activity of all proteins and genes occurs within a time interval $\triangle t$. However, it is not necessarily

true – switching of gene activity of different genes usually does not occur at exactly the same time points. There are different ways to escape this problem. One can separate different scales of reactions [7] or substitute the synchronized updates with updates time in continuous time as described by differential equations [25]. Taken together, comparison of a model assumption with experimental observations suggests that at some level of idealization and coarse-graining, a network can be viewed as consisting of discretely behaving, binary elements that can be modeled by a Boolean network.

The next section describes specific successful examples of existing Boolean networks models in systems biology.

3.3.1 Boolean network models in systems biology

After the validation of the Boolean network approach to regulatory networks, let us give a brief overview of the current state of the Boolean network models in systems biology. The rapid development of this field was triggered by new available experimental data, the amount of which is huge and growing, as it was mentioned in chapter 2. However, the available data in most cases is not expanded enough as it is needed for building such detailed models as ODE, in particular, the precise concentrations and kinetic rates are rarely known. This paves the way for developing Boolean network models.

The Boolean network method appeared to be suitable for many biological systems, therefore many Boolean network models were built in the last time. Among existing models, early examples are the work of F. Li et al. dealing with the dynamics of the budding yeast cell-cycle and by R. Albert et al. modeling the segment polarity gene network in Drosophila melanogaster.

In the first work [123], the approach of the Boolean network was applied to describing the budding yeast cell cycle, using the simplest synchronous update and

threshold Boolean functions. Applying this approach it was possible to understand how the stability of a cell state is achieved and how a biological pathway brings the cell from one state to the next one. This model will be further compared with our fission yeast cell cycle model, and dynamical properties of both models will be discussed in section 6.3.

In the second work [7], the specific biological system of segment polarity genes of Drosophila melanogaster was analyzed using a Boolean network with canalizing Boolean functions of update. In the first version of the model the synchronous update was used. In the proposed model the spatial and temporal patterns of genes expression were determined by analyzing the topology of the network. The most surprising result was the fact, that even the use of discrete dynamics neglecting many details of interactions suffices to reproduce the wild-type gene expression patterns, as well as the ectopic expression patterns observed in overexpression experiments and various mutants. The model gave new insights in understanding the crucial role of the wingless and sloppy genes and the networks abilities to correct errors in the prepattern. In both works [7, 123] the analysis was made by finding attractors, which correspond to certain biological states.

The first version of the model was further developed by introducing asynchronous update, taking into account the fact that genes do not match in step [31]. The comparison of these two versions demonstrated that the steady states of the model remain the same but in a second version oscillations are also possible. At this point it is necessary to note that in case of asynchronous update the same initial condition may lead to different steady state depending on the order of update, thereby a stochasticity is introduced into the Boolean model.

Recently, in 2006, a new, even more sophisticated version of the model was developed [32]. In the last version, which is called continuous – Boolean hybrid model, the ODE and Boolean approaches are combined together: Every node is characterized by

both – a continuous and a Boolean variable. The Boolean description is responsible for activity whereas the continuous representation corresponds to the concentration. This hybrid model predicts that transient disregulation of post-translational modification can influence as harsh as gene knockouts [32]. Another inspiring fact, already mentioned in 3.1.1 is that the dynamical behavior of the segment polarity network is *Drosophila melongaster* is determined by the network topology rather than by the exact values of the kinetic parameters.

Other examples of Boolean network models include the genetic network underlying flower development in A.thaliana [55, 145, 197], signal transduction network for abscisic acid induced stomatal closure [124] and of the mammalian cell-cycle [56].

As a result it is possible to expect that the Boolean approach can be readily applied to other gene regulatory network with relatively good characterized interactions. This approach allows to have such a view on a system as a view from an airplane on the earth, where on the one hand, you cannot see everything in much detail, but on the other hand it is nevertheless possible to observe the whole structure of the system and the main mechanisms of its functioning. This is of vital importance for understanding the main properties of the observed system. In biological systems this approach unifies qualitative observations of genes and protein interactions into a unique picture. In many cases this approach has some certain advantages comparing with differential equations approach, where we always need to know the value of all kinetic parameters which are in most cases unknown. Thus with Boolean models we can more easily perform a systematic study of possible steady states and attractors. Such models could be a good first approach to understand the interplay between the functioning of a protein-protein or protein-gene network and its topology.

The networks described above are small networks that are constructed for a certain biological process. On the other hand with a large number of molecular mechanisms involved in gene regulation have been described during last decades, it is becoming

possible to address questions about the global structure of gene regulatory networks, at least in the case of some best-characterized organisms. The verification of large Boolean network models is much easier than large ODE systems, therefore the future perspective is to build large multi-components Boolean network models that embrace a large number of components involved in regulatory mechanism. Such models would be able to give a realistic coarse-grain picture of sophisticated multi-component biological processes without problems that one meets with ODE.

3.3.2 Conclusion

Depending on a level of granularity and on amount of available data, different modeling approaches can be used. The most widespread method is ODE, which captures the time dependence of each participating component, thereby providing rather detailed description. The main challenge one meets working with ODE is finding the right kinetic constants, which in most cases are out of reach from experimental data. It is also difficult to include a large number of components using ODE, since the problem of unknown kinetic constants becomes almost not-solvable for a large differential system. This is accompanied with technical difficulties in deriving large differential equation system. The more detailed methods, as PDE and stochastic master equations, although have the highest potential in describing the nature of the process are not used very often because of the lack of data, dimensionality and technical difficulties.

The amount and quantity of available data has triggered the development of course-grained approach – Boolean networks and its different modifications such as hybrid methods. This approach has less demand on data – no kinetic parameters and no exact concentrations are needed. This approach allows to reproduce the sequential pattern of states and can be applied for many biological systems for which the exact timing course is not needed. The computer simulations of such models are also much

easier than ODE models. Due to all these reasons, Boolean network approach allows to construct models consisting of a large number of components.

Thus, having pros and cons of different mathematical approaches in mind, depending on a particular biological process, on amount of available data, and on the level of granularity, the appropriate method can be chosen.

The next three chapters describe the application of the Boolean networks approach to apoptosis and the fission yeast cell cycle which is a core of this thesis.

4. BOOLEAN MODEL OF APOPTOSIS

Chapter 4 is devoted to apoptosis, its description as a biological process with a subsequent introduction of our Boolean network model for this process. The chapter is organized as follows: The first part, 4.1 describes the role of apoptosis followed in section 4.2 by the description of the apoptosis mechanism. Section 4.3 outlines different approaches of mathematical formalization of this process, i.e. it gives an overview of existing mathematical models of apoptosis accompanied with a discussion of their strong and weak points. The last section 4.4 is devoted to our Boolean network of apoptosis and includes a description of the apoptosis network, its dynamical properties as well as a verification of the model. Finally we discuss the specific challenges facing with modeling of apoptosis.

4.1 Apoptosis

Apoptosis is a process of a programmed cell death and is an essential part of many processes of organisms e.g. development, differentiation, proliferation/homoeostasis [200], regulation and function of the immune system [63]. Apoptosis is also vital for cell termination. It occurs when under certain physical, biochemical or biological injuries, a cell cannot recover from the respective damage. The damage of apoptosis regulation causes many diseases: the lack of apoptosis (deficiently of apoptosis) is associated with cancer, viral infections and auto-immunity, the exaggeration causes heart disease, stroke, neurodegenerative disease, sepsis and multiple organ dysfunction

[63].

From a biological point of view apoptosis is a very complicated process where in the decision whether to die or not a lot of proteins and genes are involved. The balance between cell survival and death is under genetic control. The difference between apoptosis and another process of cell death, necrosis, is that during apoptosis cells regulate themselves, this is why apoptosis is often referred to a cell suicide. Once the decision to die or not is taken, the proper execution of the apoptotic program demands the coordinated activation and execution of multiple processes [76]. The next section describes the regulatory mechanism of apoptosis.

4.2 Mechanism of cell death

Apoptosis consists of four phases: initiating, decision-making, execution and clearing phases [51, 120, 233]. In the first initiating phase, the cell receives signals through different external and internal triggers. As soon as a signal has reached the cell, it starts to propagate and during decision-making phase numerous of proteins influence the spreading of the incoming signal whether blocking it or not. The third execution phase starts when the cell achieved the step after which the process is irreversible. In execution phase nuclear DNA is cleaved, the cell splits into apoptotic bodies. In final clearing phase phagocytosis takes place when the cells digest apoptotic bodies. The main challenge in apoptosis research is to comprehend the complex interactions between positive and negative regulatory proteins during the decision-making phase, which determine the fate of the cell to stay vital or die. Therefore, we will further mostly concentrate on a description of the decision-making phase and causing the initiating phase.

Initiation phase

There are a number of mechanisms through which apoptosis can be initiated in cells. All initiating apoptosis stimuli can be divided in two groups – intracellular and extracellular [63]. Extracellular stimuli include toxins, growth factors, cytokines (respond to immune system, f.e. IL-1 signaling proteins), survival factors (f.e. NGF, IL-3 proteins), activation of Granzyme [161, 177, 221] or death receptors. Activation of death receptors occurs when cells recognize damage or virus infected cells. It works as a protection mechanism to prevent damaged cells from becoming cancerous or virus-infected.

Two main family receptors that initiate apoptosis are Fas-receptor and TNF-family receptor. The Fas receptor is a transmembrane glycoprotein death receptor [34, 45, 63]. Binding FAS to Fas ligand forms death-inducing complex (DISC), which includes activation of FADD, caspase 8 and caspase 10 [169]. The Fas pathway is important in controlling the immune response. Activation of TNF receptor can initiate different biochemical pathways [86, 130] via TRADD and FADD intermediate proteins [218] . TNF may also activate transcriptional factors that are responsible to cell survival, for example, suppress apoptosis by binding to the receptor, TNFR2, which activates a protein known as Nuclear Factor kB (NF-kB), classed as an inhibitor of apoptosis protein (IAP) that prevents the execution phase of apoptosis [86]. Apoptosis can also be induced by cytotoxic T-lymphocytes using the enzyme Granzyme.

Intracellular signals include stress signal, increased intracellular calcium concentration, DNA-damage, radiation, toxins and hormones. These are the factors that lead to the activation of intracellular apoptotic signals by a damaged cell and in general they involve the mitochondria and are controlled by various bcl-2 family proteins [38].

Decision – making phase

All initiated pathways of apoptosis can be divided into two main groups – mitochondria dependent and mitochondria independent pathways [63]. If in extrinsically initiated apoptosis a coming signal is not strong enough for generating caspase signaling, the signal needs to be amplified via mitochondria-dependent apoptotic pathways. Protein Bid (Bcl-2 family) provides a connection between the caspase signaling cascade and mitochondria [125]. Bid is activated by caspase 8 and translocates to mitochondria, where together with Bax and Bak proteins induce the release of cytochrome c. Cytochrome c binds to Apaf-1, which then triggers the activation of the procaspase-9. It subsequently activates caspase 9 [1] and the executor caspases – caspase 3, caspase 7, caspase 6 and results in a cell death [63, 184].

Mitochondria independent extrinsic pathway recruits activation of procaspase-8, that triggers subsequent activation of caspase 8, which directly initiates caspase 3 followed by the death of the cell. This pathway can be inhibited by anti-apoptotic proteins p35, CRMA or FLIP. Executor caspases can be also directly activated by Granzyme B, if Granzyme B is delivered into cells by cytotoxic T lymphocytes [63].

Besides extinsic apoptotic pathways, mitochondria, as it was already mentioned above, plays a vital role in intracellular meditated apoptosis. The intracellular signals trigger changes in mitochondria membrane which result in release of cytochrome c that activates caspases. Moreover, cytochrome c also activates apoptosis-inducing factor (AIF), the endonuclease endoG and Omi [63, 122, 189, 212]. Activation of these proteins may also result in execution of apoptosis. Therefore mitochondrial proteins are very important in mediating enhancing apoptotic pathways.

However, these proteins are under control of Bcl-2 family proteins [63].

Among them Bcl-2, Bcl-Xl proteins are antiapoptotic proteins, Bax, Bak, Bid and Bad are pro-apoptotic proteins. Specific apoptosis stress signals activate particular pro-apoptotic proteins only which then interact with anti-apoptotic members of the

family. These directly control the release of mitochondrial proteins. Bcl-2, Bcl-Xl are upregulated by NF-kB [79]. NF-kB is a central regulator of innate and adaptive immune response. In apoptotic machinery it is usually described as an anti-apoptotic transcription factor, since it induces the expression of pro-survival Bcl-2 proteins. However, under certain conditions NF-kB may also contribute in apoptosis induction [63]. Besides that, NF-kB activates anti-apoptotic gene IAPs (inhibitor of apoptotic proteins). IAP family proteins (xIAP, c-IAP1, c-IAP2) directly inhibit executor caspase 3, 7, 9. It is important to notice that Smac, released from mitochondria membrane, is able to prevent inhibitor effect of IAPs. Akt-PKB kinase negatively regulates Bad, procaspase-9 and stimulates NF-kB survival pathway by activation of IkB kinase [63].

Probably the most important tumor suppressor factor is protein p53 [63, 76], which is mutated in more than the half of all known types of cancer. It is activated as a transcription factor in response to DNA damage, oncogene activation. P53 stimulates the expression of Bax, Apaf-1, FAS and represses activation of anti-apoptotic proteins, e.g. Bcl-2, Bcl-Xl, and sirvivin. On the other hand, p53-initiated pathways can be suppressed by anti-apoptotic proteins, such as growth factor which binding to receptor may result in Akt activation.

Thus, the response of cells to any of these extracellular or intracellular triggers varies depending on different factors such as the activation of positive and negative regulating apoptosis proteins, the severity of the stimulus and the stage of the cell cycle. The balance between the activation of pro-apoptotic and anti-apoptotic proteins determines the fate of a cell.

Execution phase

Most of the apoptotic pathways result in caspase activation [87, 177]. Twelve human caspases (CASP 1-12) have been described. The actions of the caspases are varied; some are endonucleases that cleave DNA, some cleave cytoskeletal proteins

and others cause a loss of cell adhesion. Caspase 1 and caspase 4 are involved in inflammation [52]. Activation of caspase 8 and 10 via death receptors typically results in activation of executor caspases. The executor caspases 3, 6 and 7 are responsible for the cleavage of the key cellular proteins, such as cytoskeletal proteins, that leads to the typical morphological changes observed in cells undergoing apoptosis.

Summary

Apoptosis is a very complicated genetically regulated process. It can be initiated by a big variety of reasons and involves many interconnected pathways that build a very complex network of interactions. Once the decision is taken, the apoptotic program switches on a coordinated activation and proper execution of the multiple sub-programmes that result in a cell destruction. However, although many of the key apoptotic proteins have been identified, there is still a lot unclear about molecular mechanisms of action and activation of these proteins.

4.3 Mathematical modeling of apoptosis

Apoptosis, as a very complicated process, consisting of a web of interconnected pathways, requires mathematical modeling for understanding its complex behavior. Before giving an overview on existing mathematical models of apoptosis, one has to notice that even though apoptosis research is a very active developing field and thousands of papers are published every year, there is still no experimental approach available at present that allows monitoring of all long-term and immediate changes of all proteins involved in this process [16].

Mathematical models of apoptosis typically use a classical ODE approach and simulate well-investigated pathways or parts of pathways, where most biochemical mechanisms are studied in much detail. As described before, this approach requires information on protein concentrations and reaction rates. For most reactions, this

information is not directly accessible, therefore in all ODE models the hardest part is to find the right parameters that will match model behavior and experimental data. As a result, most of the apoptosis models include a very limited number of proteins and are still rather abstract.

The first attempt to model apoptosis with more than 20 reactions was proposed in [61]. This model simulates two apoptotic pathways – receptor-induced activation via FAS and stress induced activation via mitochondria. Due to the dearth of information on biochemical reactions, the authors based their model on ad hoc fixed parameters, therefore its potential for understanding the regulation of apoptosis remains limited.

Four years later, a more advanced and realistic model of apoptosis induced by CD95 (TNF-R family receptor) was presented in [16], which was directly verified with experimental data. The model describes two CD95-initiated pathways – mitochondria dependent and independent. This is the first model involving large-scale apoptotic pathways. The numerical simulations of the model suggest the threshold behavior in initiating apoptosis via CD95 receptor and make the realistic predictions of the evolution of behavior of the system.

After one more year, in 2005, a mathematical model of a modular network coordinating the cell cycle and apoptosis by Aguda and Algar [37] was constructed. After analysis of the pathways linking the triggers of the cell cycle and apoptosis, authors of [37] suggested a modular organization of the pathways and proposed a corresponding kinetic model. The model represents the cellular state transitions from quiescence (nondividing) to cell cycling as well as to apoptosis pathway which becomes activated on a response of the increase of the extracellular signals. The model shows the threshold behavior between the cell cycle and switching apoptotic pathways. Although the model is able to make some predictions, it is still too abstract, therefore some behaviors of the system appear only in the model and do not correspond to experimental reports. To make this model representative, one needs to extend it to

a realistic network that includes the key components of the cell cycle and apoptosis.

The threshold behavior between cell survival and death are also suggested in paper [223], where a series of kinetic models that describe the cross talk between p53 and Akt proteins are built (the biggest model consists of 8 proteins). These models demonstrate robustness of the bistable behavior and the authors argue that this is a mechanism for a cellular survival-death switch. They suggest the following reasons, why bistability is an important property of a cellular switch between survival and death: A bistable region ensures a range of parameter values within which the switch can be controlled by external perturbations or signals from other pathways; As a consequence of the positive feedback loop between p53 and Akt, the apoptotic threshold depends on both states of these proteins. The bistable switch-like behavior is further confirmed in another model of caspase activation [50] and verified with experimental data.

Summing up, there are several models of some modules and parts of apoptosis, but there is a dearth of an integrating model of apoptosis that would allow to understand the control mechanisms of the process and to make predictions. The reason for this is that for modeling apoptosis with the ODE approach used in all previous models, the detailed knowledge on reactions and concentrations are needed, as it has been discussed in 3.1.1. This approach is impossible to apply for bigger parts of apoptosis because of dimensionality: Since in most cases kinetic parameters are not known the only way to find them is to fit the results with experimental data. This problem becomes incredibly difficult with increasing the number proteins. On the other hand the currently existing models suggest that different modules of apoptosis play a role of switching elements. This brought us to the idea of building a general Boolean network model.

4.3.1 Boolean network model of Apoptosis

In this subsection we build a Boolean network model of apoptosis for a human cell and present the results of its simulation.

Due to the reasons explained in the previous subsection, we decided to use the Boolean network approach for creating a general model of apoptosis. This approach abstracts (as it has been explained above) from the details of biochemical reactions approximating all reactions to activation and inhibition, as well as reduces the different levels of proteins concentrations to high/low concentrations (0/1 state) that eliminates the most difficulties of ODE models.

Therefore, after extensive literature and biochemical data bases studies [17, 89, 121, 140, 167, 176, 194, 232], we obtained the following scheme of interactions for initiating, decision-making and execution phases, integrated in Fig. 4.1. In Fig. 4.1. proteins are represented by nodes except intracellular signals of apoptosis – DNA-damage, Ca++ signalling, ER-stress, Stress-signal and the final node *Apoptosis*, which can be activated after a cascade of interactions between other proteins (nodes). Activation of the *Apoptosis* node denotes a death of a cell. The network in Fig. 4.1 includes intracellular (DNA damage, ERstress, increase in calcium concentration) and extracellular (IL-1, survival factors – NGF, IL-3 proteins, Granzyme B or death receptors (TNF and FAS)) induced pathways, described in section 4.2.

Following the Boolean network concept, all nodes assigned a binary value, $S_i(t) \in \{0, 1\}$, denoting whether the protein is present or not. It has to be noticed, that there are two types of nodes in the system: 1) Independent nodes (green rectangles) 2) Dependent nodes (blue and yellow rectangles). Independent nodes are the nodes that do not have incoming links, and ON/OFF values are assigned *a priori*. Independent nodes in most cases correspond to initiating apoptosis signals (IL-1, NGF, IL-3, Granzyme B, TNF, FAS, TRAIL, DNA damage, ERstess, Ca++ signalling). Besides them, there are some regulating proteins – FLIP, CRMA, p35, smac, Omi, caspase4

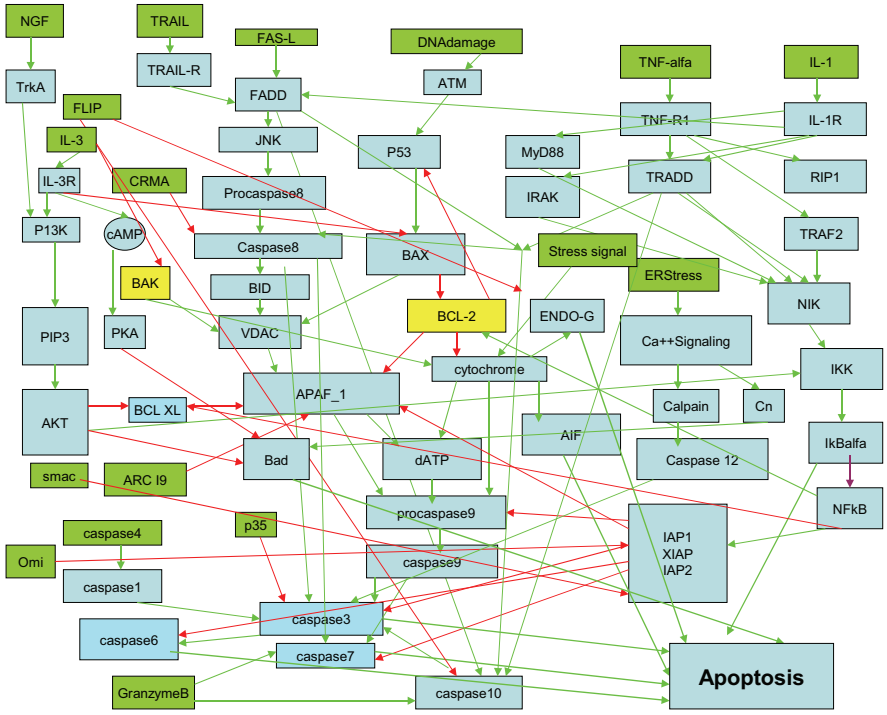

Fig. 4.1: Schematic diagram of interactions in apoptosis. Green/blue and yellow rectangles are independent/dependent nodes correspondingly. Green/red links are activating/inhibiting links correspondingly.

that are not internally controlled by other proteins in the frames of our model and therefore modeled as independent nodes as well.

The vector of ON/OFF values of the independent nodes is an initial condition of the system ($S(0)$). It explicitly determines the output of the system, since the system is deterministic and the update function for every node is known and determined. All dependent nodes change their values following update functions, described below.

Interactions between proteins are represented by links. All types of interactions are reduced to Activation/Inhibition (green/red links in Fig. 4.1 correspondingly). As in [7] we use canalizing Boolean function, as the simplest possible rule from available data. Nodes which are active at some time step and having an active/inhibiting

outgoing link, will send a positive/negative signal to a receiving node at the next time step. The special case are yellow rectangle nodes. These are the nodes that do not have any positive input signal (nodes: BAK and BCL-2). These two nodes are considered as independent nodes in case they do not receive negative signals and as dependent nodes in the opposite case. Also incoming positive links gathering TRADD, FADD and caspase 8 can transmit positive signals only if these nodes are active and FLIP is not active.

All nodes are updated synchronously. The apoptosis Boolean model is simulated starting from all possible initial states – combinations of active/inactive independent nodes. It is known that apoptotic pathways are not activated until they received a triggering signal [63], that is we start our simulations with all dependent nodes OFF at the first time step. A dependent node becomes active at some time step if it received only positive signals and no negative (no inhibiting) signals. If no signals were coming, the node keeps its state. Otherwise, in case of at least one negative signal, the node switches to OFF-state. These rules are supported by bistable, switch-like decision behavior confirmed by [37, 219, 223], the authors of which argue that different modules of apoptosis have two stable steady states and depending on initial conditions or incoming signal converge to one of them.

4.3.2 Results

We run the Boolean network model of apoptosis for all possible initial conditions and the results of our simulations suggest that in 86% the *Apoptosis* node is activated which corresponds to the death of the cell. Therefore the results of our Boolean network model argue that apoptosis can be a sufficiently robust irreversible process. As the next step we compared the network dynamics to 'null' model of random networks with the same number of inhibiting and activating links and the same updating rules. Our simulations (averaged over 1000 random networks) show the rate of apoptosis is

about 35%. This suggest that initial network of apoptosis has very special topology that provides the certain dynamical properties.

We also tested how the rate of apoptosis changes when we delete important proteins, i.e. nodes: P53, TNF-alfa, ER-stress, CRMA, P35, smac, IL-3, Caspase 4. Our results indicate that deleting one of following nodes – TNF-alfa, CRMA, P35, ER-stress, stress-signal, increases the rate of apoptosis to 93% , whereas deleting one of CRMA, smac, P53, caspase 4, IL-3 nodes reduces it to 83% . In experiments one observes qualitatively similar behavior: Decrease in expression of CRMA, smac, P53, caspase 4, IL-3 as well as disactivation of ER-stress and stress signal, reduces the probability of apoptosis, whereas the descrease in expression of TNF-alfa, CRMA, P35 increases it [40, 90, 91, 224].

4.3.3 Conclusion

Thus, we have constructed a Boolean network model of apoptosis for human cells. The results of the simulation suggest that as one of the apoptosis pathways has been activated, in spite of anti-apoptotic proteins and survival signals that can withstand, in most cases the cell dies. The model was verified via deleting a number of important proteins and observing the corresponding changes in apoptosis rate. The predictions of our model on the influence of different proteins can be useful in developing new drugs: As soon as we know which protein can be activated/inhibited for increasing/decreasing of apoptosis rate, the appropriate control of the process can be performed.

However, it is difficult to make a complete verification of our Boolean network model of apoptosis, because there is no experimental data that allows to monitor the behavior of more than a single pathway, grasping the complex behavior of all pathways in parallel. Another challenge that we faced working on Boolean apoptosis model is a large variety of reasons that trigger this death machinery, which makes

the state space of initial conditions too big for exact verification. In some cases there is still not enough information on interactions between proteins. For these reasons we looked for another biological process that is easier to verify and has smaller set of initial states, i.e. which is from topological point of view a closed module with a small number of incoming signals. The fission yeast cell cycle totally satisfies these conditions and therefore we will concentrate on this process in the rest of the thesis.

5. CELL CYCLE OF FISSION YEAST

This chapter is devoted to the fission yeast cell cycle – a description of known biological details of the process and previous existing models of it. The first section starts with a general introduction of the cell cycle and is followed by a specific details of the fission yeast cell cycle. In the second section the existing ODE models of the fission yeast cell cycle are presented and discussed.

5.1 Cell cycle

The process responsible for replication of DNA and other cellular components, resulting in a cell doubling, is a cell division cycle or also often named 'cell cycle' for short. The cell cycle is vital for growth, development and for keeping living organisms alive. This process represents itself a series of events, which are highly ordered. During cell division cells undergo several discrete transitions. A cell cycle transition is an irreversible change of state in which a cell moves its activity from executing one set of processes to a different set of processes. The current research of the cell cycle focuses on the question, how these transitions are regulated that they occur at a certain time and in a special order of events. The particular interest to the cell cycle research field is due to the fact that the disregulation and uncontrolled proliferation through the cell cycle causes cancer [2].

The eukaryotic cell cycle consists of four phases G1-S-G2-M. The transition to the next phase occurs only after appropriate completion of the previous one.

During the first phase G1 (G indicates Growth), the cell grows and specific enzymes are synthesized which are required in the next S phase for DNA replication. At the second phase S (S indicates Synthesis), DNA is synthesized and chromosomes are replicated. This phase is completed when each chromosome has two sister chromatides. It is followed by G2 (G2 indicates 'Gap'), which continues until the cell enters mitosis. This phase is necessary for synthesis of significant proteins essential for mitosis. Inhibition of this synthesis prevents the cell from entering mitosis [2].

The final phase M (M indicates Mitosis) consists of two connected processes – mitosis and cytokinesis. In mitosis the cell's chromosomes are separated between two daughter cells, in cytokinesis the cell cytoplasm divides and forms two cells. Eventually, after M phase, the cell enters G1 again, thereby completing a cycle [2].

The cell cycle has two checkpoints – G1/S and G2/M, which are used for monitoring and regulating the processes of the cell. The purpose of these checkpoints is to prevent further progression of cell cycle if some processes were not completed or went wrong, i.e. if DNA is damaged, the checkpoint stops the cell cycle until DNA is repaired or in case the reparation is not possible the checkpoint targets the cell to apoptosis.

The next subsection gives more detailed biological knowledge on the biochemical reactions during the cell cycle.

5.1.1 Regulation of cell cycle

Paul Nurse [156] proposed that in different organisms the cell cycle is controlled by a common set of proteins interacting with each other by a common set of rules. However, every particular organism has its own specific set of proteins and interactions. This specific set determines exactly which parts of the common machinery are performing in a certain organism depending on a developmental stage of it. This subsection describes briefly a general scheme of the cell cycle – the main participants and the

relations between them. In the next subsection the particular mechanisms of the fission yeast cell cycle are represented.

The cell cycle is controlled via a complex network of interactions between three types of proteins: Cyclin-dependent kinases, cyclins and inhibitors of CDK/cyclin complexes. Cyclins and CDKs can "work" only together, since cyclins have no catalytic activity and CDK are active only with a bound cyclin. Cyclin/CDK complexes are important for catalyzing phosphorylation of proteins essential for cell cycle progression. During different stages of the cell cycle various cyclins are synthesized, which together with CDK form different complexes. These complexes regulate different phases of the cell cycle and therefore are called G1-, G1/S, S and M-CDK.

An external signal initiates G1 cyclin/CDK complexes which start to prepare the cell for S phase – activate transcription factors of S cyclins and of enzymes required for DNA replication and degradate inhibitors of S phase. The activity of G1/S-CDK increases. Then S-CDKs inactivate inhibitors of CDK and phosphorylate different proteins, initiating S phase. It is followed by DNA replication and self-inactivation of S/G1-CDKs. In G2 phase, the M-CDKs start to accumulate which brings the cell to mitosis. M phase-CDKs trigger a cascade of protein phosphorylation and activation of 'Anaphase-Promoting Complex' (APC). This complex is essential for separation of the duplicated chromosomes. APC is antagonist to CDK: This complex targets cyclins for degradation, removal of which is necessary for exit from mitosis. CDK activity is low, APC activity is high in G1 phase and vice versa in S-G2-M phases. Therefore, during the cell cycle CDK activity varies periodically as a result of an antagonism between CDK and APC. Thus, "to understand the molecular control of cell reproduction is to understand the regulation of CDK and APC activities" [205].

In the next section the particular details of the fission yeast cell cycle are introduced.

5.2 Fission yeast cell cycle

The yeasts are unicellular eukaryotic organisms which play a big role for understanding the main biological mechanisms. For discoveries in cell cycle regulation of yeast in 2001 Paul Nurse, Timothy Hunt and Lalend Hartwell received the Nobel Prize for Physiology or Medicine [2]. Paul Nurse discovered and analyzed enzymes called 'Cyclin-dependent kinases' (CDKs). Timothy Hunt detected proteins called 'cyclins'. Leland Hartwell discovered the genes that are responsible for cell cycle control, including 'start' genes that are vital for starting S phase.

The particular interest for the yeast cell cycle is due to the fact that it is similar to the cell cycle in human: Basic mechanisms of DNA replication, recombination, cell division and metabolism share similar features. On the other hand, yeasts are easy to treat in lab and to explore using different experimental techniques. Therefore they are well studied organisms. Many cell cycle proteins important in humans were first detected by exploring their homologues in yeast.

There are two yeasts – budding (Saccharomyces cerevisiae) and fission (Schizosaccharomyces Pombe) that are usually investigated. The genome of the first one was sequenced in 1996 and has been widely used as a modeling organism. Fission yeast was sequenced 6 years later and started to be used as a modeling organism relatively recently. This organism is of high interest in the scientific community because it has many gene homologues to human diseases genes, as, for example, diabetes and cystic fibrosis and heterochromatin genes. For these reasons and also since the fission yeast cell cycle is well understood in terms of ODE models, we choose it further as a core process of our work. In section 6.3 we will compare the mechanisms of fission and budding yeasts cell cycles.

Let us first describe the specificity of the fission yeast cell cycle. The biochemical reactions forming the network that controls the fission yeast cell-cycle have been

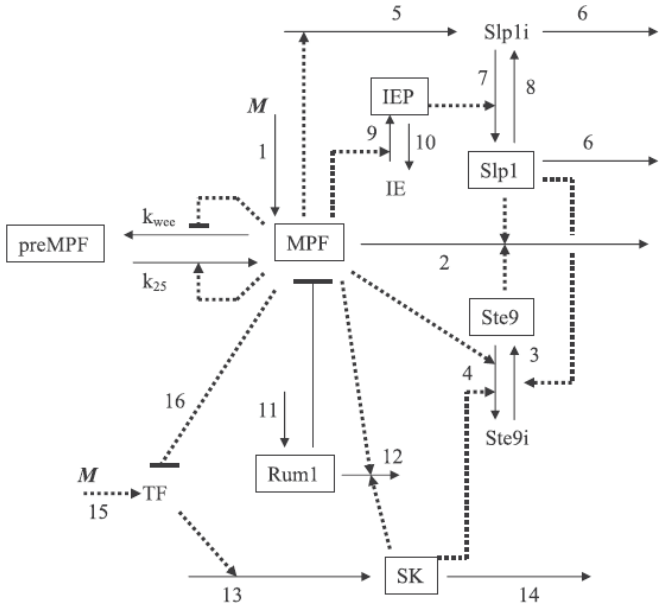

Fig. 5.1: Detailed steps of the Novak-Tyson model of the fission yeast cell cycle [154]. The figure is redrawn from [154]

studied in detail over the last years [28, 42, 97, 129, 137, 154, 166, 213, 231]. The schematic diagram is shown in Fig. 5.1. The process has two irreversible points – START and FINISH. START corresponds to start of DNA replication, FINISH refers to exit from mitosis.

The fission yeast cell cycle has only one essential CDK – Cdc2, which, depending on a certain cyclin it binds to, can initiate S and M phases. There is an antagonism between cyclin/CDK complex Cdc13/Cdc2 and APC via Ste9 and Slp1 and Rum1 proteins. Activity of Cdc13/Cdc2 can be inhibited by Rum1 as a Cdc2 inhibitor (process 1 in Fig. 5.1). On the other hand, active Cdc2 can phosphorylate Rum1 resulting in Rum1's degradation (process 2). Ste9/APC degradates Cdc13 (process 3), whereas Cdc2/Cdc13 inhibits Ste9/APC by phosphorylation (process 4). Therefore, complex Cdc13/Cdc2 cannot be in active form parallel with with Ste9/APC or Rum1. Cdc2/Cdc13 has high activity in S-G2-M phases, when APC has low activity and vice versa in G1 phase.

There are 'helper' molecules, Slp1, Wee1, Mik1, Cdc25 and Start Kinases, that are involved in antagonisms between APC and cyclin/CDK complex (processes 5, 9 and 10, in Fig. 5.1). Proteins Slp1, Wee1 and Mik1 help APC to deactivate Cdc2/Cdc13. Start kinases, SK (Cdc2/Cig1, Cdc2/Cig2, Cdc2/Puc1) can inactivate Rum1 (process 6) and Ste9/APC (process 7). However, Cdc13/Cdc2 inhibits SK activity by phosphorylation of transcription factor for SK (process 8). Process 12 indicates many processes triggered by Cdc2 to perform the START and G2/M transitions, i.e. phosphatase Cdc25 that positively regulates Cdc2/Cdc13 and Wee1/Mik1 that negatively regulates Cdc13/Cdc2. The oscilations of Cdc2 activity are provided by a negative feedback loop which consists of processes 9, 5, 3 and processes 8, 6,1 together with 8, 7, 3.

The formalization of these processes in terms of differential equations approach is described in the following section.

5.3 ODE models of Fission Yeast Cell Cycle

As a complex process, fission yeast cell cycle cannot be understood by only intuitive comprehending as already explained in chapter 1. Mathematical models have to be applied to analyze and predict the behavior of the biochemical network forming fission yeast cell cycle. The groups of Novak and Tyson (NT) have been collaborating for many years to create series of fission yeast cell cycle models [153, 154, 191, 204, 205]. Although Novaks and Tysons groups have published various versions of their fission yeast cell cycle model, we choose the most widespread version of the model [154] as often cited in textbooks and further used in this thesis in chapter 8 to show a limiting transition between ODE and Boolean networks models. We explain this model in detail to illustrate the formulation of the dynamical equations, computer simulations and bifurcation analysis. For other descriptions and more analysis of the fission yeast

cell cycle model, see [204, 205]. The details of the mechanism that correspond to Fig. 5.1 are converted into a set of dynamical equations

$$\frac{d[Cdc13_{T1}]}{dt} = k_1 M_1 - (k_2' + k_2''[Ste9_1] + k_2'''[Slp1_1])[Cdc13_{T1}] \tag{5.1}$$

$$\frac{d[preMPF_1]}{dt} = k_{wee_1} k_0 (k_0''[Cdc13_{T1}] - [preMPF_1]) - \tag{5.2}$$
$$-(k_{25} k_0'''[preMPF_1] - k_2' + k_2''[Ste9_1] + k_2'''[Slp1_1])[preMPF_1]$$

$$\frac{d[Ste9_1]}{dt} = (k_3' + k_3''[Slp1_1]) \frac{1 - [Ste9_1]}{J_3 + 1 - [Ste9_1]} - \tag{5.3}$$
$$-(k_4'[SK_1] + k_4[MPF_1] \frac{[Ste9_1]}{J_4 + [Ste9_1]}$$

$$\frac{[Slp1_{T1}]}{dt} = k_5' + k_5'' \frac{[MPF_1]^4}{J_5^4 + [MPF_1]^4} - k_6[Slp1_{T1}] \tag{5.4}$$

$$\frac{[Slp1_1]}{dt} = k_7[IEP_1] \frac{[Slp1_{T1}] - [Slp1_1]}{J_7 + [Slp1_{T1}] - [Slp1_1]} - k_8 \frac{[Slp1_1]}{J_8 + [Slp1_1]} - \tag{5.5}$$
$$-k_6[Slp1_1]$$

$$\frac{d[IEP_1]}{dt} = k_9[MPF_1] \frac{1 - k_9'[IEP_1]}{J_9 + 1 - k_9'[IEP_1]} - k_{10} \frac{k_9'[IEP_1]}{J_{10} + k_9'[IEP_1]} \tag{5.6}$$

$$\frac{d[Rum1_{T1}]}{dt} = k_{11} - (k_{12} - k_{12}'[SK_1] + k_{12}''[MPF_1])[Rum1_{T1}] \tag{5.7}$$

$$\frac{d[SK_1]}{dt} = k_{13}[TF_1] - k_{14}[SK_1] \tag{5.8}$$

$$\frac{dM}{dt} = \mu M \tag{5.9}$$

$$[TF_1] = G(k_{15} M, k_{16}' + k_{16}''[MPF_1], J_{15}, J_{16} \tag{5.10}$$

$$k_{wee_1} = k_{wee}' + (k_{wee}'' - k_{wee}') G(V_{awee}, V_{iwee}[MPF_1], J_{awee}, J_{iwee}) \tag{5.11}$$

$$k_{25_1} = k_{25}' + (k_{25}'' - k_{25}') G(V_{a25}[MPF_1], V_{i25}, J_{a25}, J_{i25}) \tag{5.12}$$

$$[MPF_1] = \frac{(k_{17}[Cdc13_{T1}] - k_{17}'[preMPF_1])}{([k_{17}[Cdc13_{T1}])} \times \tag{5.13}$$
$$\times \frac{(k_{17}'''[Cdc13_{T1}] - k_{17}''[Trimer]}{k_{17}'''[Cdc13_{T1}]}$$

$$Trimer = \frac{k_{18}[Cdc13_{T1}][Rum1_{T1}]}{\sigma + \sqrt{\sigma^2 - k_{18}'[Cdc13_{T1}][Rum1_{T1}]}} \tag{5.14}$$

$$\sigma = k_{19}'[Cdc13_{T1}] + k_{19}''[Rum1_{T1}] + K_{diss} \tag{5.15}$$

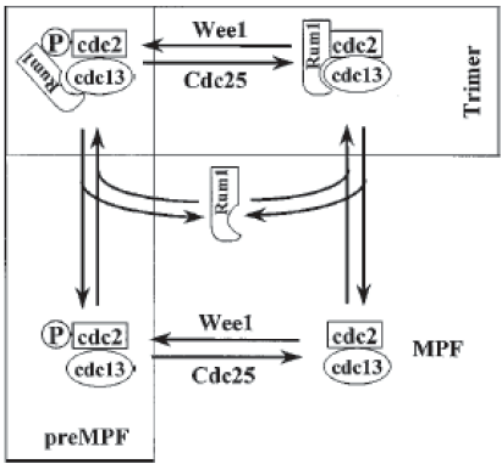

Fig. 5.2: Different protein complexes involving Cdc13/Cdc2. Image is taken form [2]

where Goldbeter-Koshland (G-K) [154] function has the following general form:

$$G(a,b,c,d) = \frac{2ad}{b-a+bc+ad+\sqrt{(b-a+bc+ad)^2-4ad(b-a)}}. \tag{5.16}$$

Growth Cycle

Mass (M), later in bifurcating analysis of the model used as a controlling parameter, is modeled by exponential growth (5.9). Cell mass grows until the activity of MPF decreases rapidly below 0.1 (threshold chosen by [154] for reproduction the known data from experiments) at the end of mitosis, then M is divided by two [2, 154]. In all models of Tyson and Novak M triggers the synthesis of Cdc13. However, in fact it does not have direct mechanistic coupling, which is a main disadvantage of these models. The transcription factor TF is also very sensitive to increase in mass, as it is modeled with Goldbeter-Koshland [72] function G.

MPF Dynamics

Cdc13T denotes a total concentration of Cdc13/Cdc2 complexes, including Rum1-Cdc13/Cdc2 trimers [2]. The first term, k_1M on the right hand side (r.h.s.) of (5.1)

assumes that the Cdc13 is synthesized with a speed proportional to the cell mass M. The rest terms of r.h.s. refer to degradation of Cdc13 due to Ste9 and Slp1. The schema of different protein complexes involving Cdc2/Cdc13 is shown in Fig. 5.2, where Cdc2/Cdc13 is presented in two forms – tyrosine-phosphorylated form - preMPF and with removed phosphorylation MPF. In equation (5.2) the first term describes the rate of dephosphorylation of preMPF. The following three terms in (5.2) correspond to degradation of preMPF. It is important to note about the second term k_{wee}. This term is proportional to a Golbeter-Koshland function (5.16), whose second argument is a function of [MPF]. Therefore, increasing in [MPF] concentration decreases k_{wee} forming a negative feedback loop. Thus, k_{wee} represents the mutual antagonism between Wee1 and MPF. Analogical explanation can be given to k_{25}: MPF and Cdc25 form a positive feedback loop. Equations (5.14) and (5.15) assume momental equilibrium between Rum1 and Cdc13.

Dynamics of the FINISH Module (Slp1/APC and IEP)

Slp1 is represented by two equations (5.4) and (5.5), corresponding to total concentration and activity of Slp1 respectively. In (5.4) the first term refers to Slp1 synthesis independent from MPF. The second term in (5.4) has a form of a Hill-type equation, which provides a switch-like behavior for Slp1, regulated by MPF. The last term of (5.4) and (5.5) is degradation of Slp1. The Fig. 5.1 illustrates equation (5.5): The steps 7 and 8 have Michaelis-Menten kinetics. The last term represents degradation [2].

The enzyme IEP (5.6), first proposed by Novak and later revealed in experiments provides the delay essential for the chromosomes to align with metaphase plane before they are separated in anaphase [2].

G1/S Module

The concentration of Ste9, inhibiting factor of Cdc2/Cdc13 evolves according to (5.3). The first term on the r.h.s. represents both forms of Ste9: Slp1 – independent

and Slp1-dependent [2]. Deactivation of Ste9 with a help of SK and MPF is given in the second term.

Equation (5.7) models the concentration evolution of another antagonist of Cdc2/ Cdc13/Rum1. The first term in (5.7) assumes constant synthesis of Rum1. The second term represents three ways of degradation: Self-degradation, SK and MPF dependent pathways. SK kinase concentration evolves according to (5.8).

Computer Simulations

The numerical solutions of the differential equations (5.1-5.15) are shown in Fig. 5.3 A. The kinetic parameters used for simulations correspond to the wild-type fission yeast. Every next cycle starts with almost zero MPF activity, high activity of TF, which triggers SK (step 13 in Fig. 5.1). The following step is G1/S transition, during which Ste9 and Rum1 are inhibited by SK and the activity of MPF increases to the intermediate level.

Then as soon as part-inhibition of MPF is removed by inhibition of Wee1 and activation of Cdc25, the activity of MPF increases to the maximum level and the cell enters G2/M transition. Entering mitosis, MPF activity is high and after a time delay Slp1/APC triggers the degradation of Cdc13 (steps 2, 7, 9 in Fig. 5.1). After that Ste9/APC gains the activity (step 3, Fig. 5.1) and accelerates degradation of Cdc13 causing the transition.

The cell mass grows from 1 to 2 and one has to notice that periodicity is imposed by manual dividing M by 2 at the end of mitosis, when MPF falls below the value 0.1.

Phase-Plane and Bifurcation Analysis

NT models are analyzed by bifurcation theory using one- and two-dimensio-nal bifurcation diagrams. Since the mass of the cell is considered as a main driving force for progression through the cell cycle, the authors [154] choose it as a bifurcation parameter. The response of the system is detected by changes in Cdc2/Cdc13 activity

(which is modeled by variables Cdc13T, MPF), which is small in G1, intermediate in S/G2 and maximal in M phase, thereby it distinguishes the phases. Different cell cycle phases are related to different attractors. Transitions between them are referred to bifurcations.

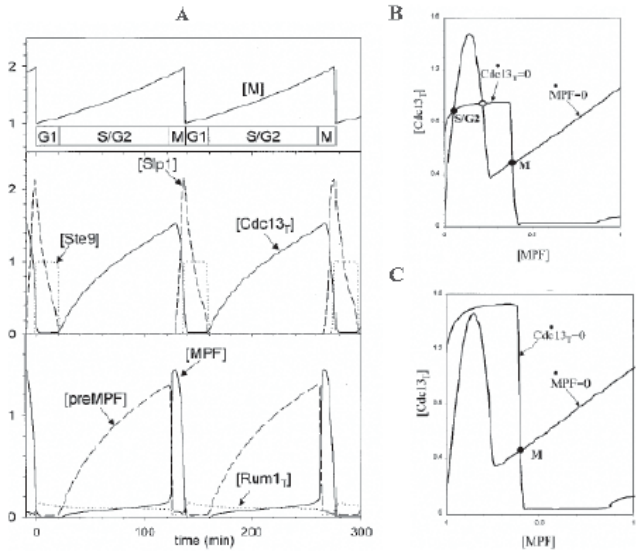

Fig. 5.3: (A) Numerical simulations of the Novak-Tyson model defined in (5.1-5.15). Figure is taken from [154]. The parameter values are listed here (all constants have units of min.1 except the Jis and k_{diss} which are dimensionless): $k_1 = 0.03$, $k_2 = 0.03$, $k_2' = 1$, $k_2'' = 0.1$, $k_3 = 1$, $k_3' = 10$, $J_3 = 0.01$, $k_4 = 2$, $k_4' = 35$, $J_4 = 0.01$, k_5= 0.005, $k_5' = 0.3$, $k_6 = 0.1$, $J_5 = 0.3$, $k_7 = 1$, $k_8 = 0.25$, $J_7 = J_8 = 0.001$, $k_9 = 0.1$, $k_{10} = 0.04$, $J_9 = J_{10} = 0.01$, $k_{11} = 0.1$, $k_{12} = 0.01$, $k_{12}' = 1$, $k_{12}''' = 3$, $k_{diss} = 0.001$, $k_{13} = 0.1$, $k_{14} = 0.1$, $k_{15} = 1.5$, $k_{16} = 1$, k_{16}'= 2, $J_{15} = J_{16} = 0.01$, $V_{awee} = 0.25$, $V_{iwee} = 1$, $J_{awee} = J_{iwee} = 0.01$, $V_{a25} = 1$, $V_{i25} = 0.25$, $J_{a25} = J_{i25} = 0.01$, k_{wee} = 0.15, k_{wee}'= 1.3, $k_{25}' = 0.05$, $k_{25}''' = 5$, $\mu = 0.005$. (B) Cdc13T and MPF nullclines for M = 1 (newborn cells), and (C) for M = 1.6 (cells that just passed the G2/M transition) • stable steady state; ◦ unstable steady state.

Fig. 5.3 shows a one-dimensional bifurcation diagram of the fission yeast cell cycle. New born cells have low Cdc2/Cdc13 activity. As the cell grows, it passes the first saddle bifurcation point: G1 steady state disappears, the cell goes through the irreversible S/G2 transition and the activity of Cdc2/Cdc13 increases to the

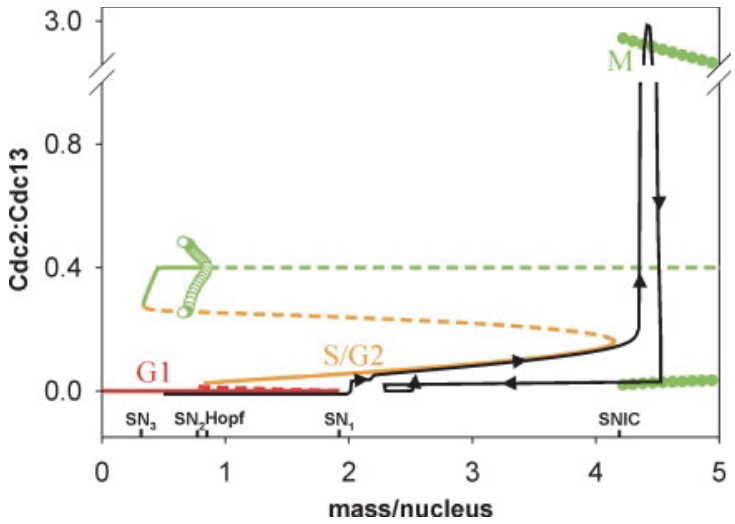

Fig. 5.4: Bifurcation diagram for wild-type cell cycle. Recurrent states of Cdc2:Cdc13 activity are plotted against the mass/nucleus ratio of the cell. Image is taken from [206]

intermediate level. At some point the cell mass becomes so large that S/G2 steady state is removed by infinite-period oscillations. The activity of Cdc2 reaches its maximum (corresponds to MPF in NT model), it drives the cell into mitosis. After some time Cdc13 is degradated by APC, therefore the activity of MPF also falls. The drop of MPF activity is a signal for a cell to divide, M is divided by factor 2 and the system is back to G1 steady state.

5.4 Conclusions

Thus, Novak and Tyson (NT) built a set of ODE models of the fission yeast cell cycle and investigated them using bifurcation analysis. The transitions between cell cycle phases are assumed to be representative by bifurcations. All NT models assume autonomous oscillations of Cdc2/Cdc13 activity, which drives the processes during the

cell cycle. The topological analysis of the cell cycle network revealed vital feedback loops.

Other ODE models of the fission yeast cell cycle are focused on a different aspect of a process. The NT models simulate not only wild-type, but a number of mutations. These are discussed in chapter 7 and compared with corresponding mutations in our Boolean network model of the fission yeast cell cycle, built in the next chapter.

The most challenging part in creating these differential equation models was finding the right kinetic constants which are mostly not available. In the next section we construct a Boolean network model for the fission yeast cell cycle, in which no parameters enter except the structure of the regulatory circuitry. This model will be able to reproduce the known activity sequence of regulatory proteins without accurate reproduction of timing.

6. BOOLEAN MODEL OF FISSION YEAST CELL CYCLE

In the following, a Boolean model of the fission yeast cell cycle will be outlined. The chapter is also in context with section 3.3.1: We compare our Boolean model of the fission yeast cell cycle with a previous existing model of the budding yeast cell cycle. In particular the topologies and dynamical properties are considered.

6.1 The fission yeast cell cycle network

Here we perform the Boolean network model that we built for the fission yeast cell cycle. First, we start a construction of our model with formalizing the known information about protein interactions described in subsection 5.2. We give a full compilation of the network of key-regulators of the fission yeast cell cycle network in Table 6.1, corresponding to our current knowledge as given in [154, 191, 205]. Also our translation into an interaction graph with activating and inhibiting links is given in the Table 6.1, which is the starting point for our discrete dynamical network simulation of this network.

Since the mechanism of activation of negative Cdc2/Cdc13 regulators is unknown, the authors of [191] assumed a similar mechanism to budding yeast. In [191] Slp1/APC degrades a hypothetical inhibitor of PP which helps PP to become active. We assume that Slp1/APC directly activates PP. Following [154] the helper molecules, such as Start Kinases (SK) are inhibited, otherwise they prevent the final transition and returning to G1 stationary state. This is why in Boolean model of the cell cycle

helper molecules - Start Kinesis (SK), Slp1 and PP have self-inhibiting links. We also represent Wee1/Mik1 by one node, since they have similar functions.

We focus on a case where all checkpoints are off except the checkpoint of the cell size. Also in the model the change in the rate of DNA replication is neglected.

Let us in the next section define the discrete dynamics that we will simulate on this graph.

6.1.1 A discrete dynamical model of the cell cycle network

We assume proteins to be the nodes of the network and assign a binary value $S_i(t) \in \{0, 1\}$ to each node i, denoting whether the protein is present or not (due to different possible biochemical mechanisms, as, e.g., gene expression of a corresponding protein, or fast biochemical reactions as phosphorylization). The interactions between the nodes, as compiled in Table 6.1, are denoted as links, see Figure 6.1.

We do not quantify any interaction strength, except whether a link is present or not, and whether it is activating or inhibiting. Again, different biochemical mechanisms are subsumed under this simplified picture, as, e.g., transcriptional regulation, or faster enzymatic interactions. We use threshold Boolean update rules as assuming a threshold mechanism of reactions, supported by previous investigations (see section 3.3), i.e. the state of the protein depends on its initial state. Here, as it has been explained in 3.3, i.e. the state of the protein depends on its initial state, in case when activating incoming signals exactly coincide with a corresponding to the node threshold: If the node was active in the previous time step it remains active and vice versa.

The states of the nodes are updated (in parallel) in discrete time steps according

Parent node	Daughter node	Rule of activation (comments)	Rule of inhibition (comments)
Start node	Kinases (SK): Cdc2/Cig1, Cdc2/Cig2, Cdc2/Puc1	Start node works as an indicator of mass of the cell and activates Starter Kinases (SK): Cdc2/Cig1, Cdc2/Cig2, Cdc2/Puc1, +1 [191].	
SK	Ste9, Rum1		Phosphorylate, thereby inactivate, -1 [191, 205]
Cdc2/Cdc13	Cdc25	Cdc25 is phosphorylated thereby activated, +1 [191].	
Wee1, Mik1	Cdc2/Cdc13*		Phosphorylate, inactivating, -1 [191]
Rum1	Cdc2/Cdc13		Binds and inhibits activity, -1 Cdc2/Cdc13 [191].
Cdc2/Cdc13	Rum1		Phosphorylates and thereby targets Rum1 for degradation. -1 [191, 205]
Ste9	Cdc2/Cdc13		Labels Cdc13 for degradation [205, 191], -1.
Cdc2/Cdc13*, Cdc2/Cdc13	Slp1	Highly activated Cdc2/Cdc13 activates Slp1, (Cdc2/Cdc13* and Cdc2/Cdc13 both active) [154, 191]+1.	
Slp1	Cdc2/Cdc13		Promotes degradation of Cdc13, thereby the activity of Cdc2/Cdc13 drops -1 [191]
Slp1	PP	Activates, +1 [191]	
PP(Unknown phosphatase)	Ste9, Rum1, Wee1, Mik1	Activates Rum1, Ste9, and the tyrosine-modifying enzymes (Wee1, Mik1, [191], +1	
Cdc25	Cdc2/Cdc13*	Cdc25 reverses phosphorylation of Cdc2, thereby Cdc2?Cdc13* becomes active, +1 [191, 154]	
Cdc2/Cdc13	Ste9		inhibits -1 [154]
PP	Cdc25		inhibits -1[191]
Cdc2/Cdc13	Wee1, Mik1		inhibits -1 [154]
Cdc2/Cdc13*	Rum1, Ste9		inhibits -1[154]

Tab. 6.1: The rules of interaction of the main elements involved in the fission yeast cell cycle regulation.

to the following rule:

$$S_i(t+1) = \begin{cases} 1, & \sum_j a_{ij} S_j(t) > 0, \\ 0, & \sum_j a_{ij} S_j(t) < 0, \\ S_i(t) & \sum_j a_{ij} S_j(t) = 0, \end{cases} \tag{6.1}$$

where $a_{ij} = 1$ for an activating interaction (green link) from node j to node i, and $a_{ij} = -1$ for an inhibiting (red) link from node j to node i, and $a_{ij} = 0$ for no interaction at all. This definition follows closely the approach in [123]. The dramatic simplification steps in constructing this model consist in not differentiating between absolute values of interaction strengths on the one hand, and not distinguishing between the different time scales of the biochemical interactions involved on the other. This corresponds to dropping all biochemical parameter values, time constants as well as binding constants, from the differential equation models, in particular dropping 48 kinetic constants used in NT ODE model introduced in the previous chapter. As we will see below, dynamical models on networks can be built to be insensitive to these parameters, provided that the interaction topology has certain properties.

Two of the ten nodes included in the model exhibit a slightly different activation behavior, which we account for by a non-zero activation threshold. Cdc2/Cdc13*, the highly activated form of the complex Cdc2/Cdc13, has to be actively maintained by a positive regulatory signal, therefore $\theta = 1$ for this node. The second special rule is to add self-activation (corresponding to adding a negative activation threshold θ = -1) to the node Cdc2/Cdc13, as it is otherwise not positively regulated. The biological motivation for this rule is the following. Cdc13 is constantly synthesized and after synthesis it immediately associates with Cdc2 [153]. Intracellular concentration of Cdc2 does not vary throughout the cell cycle [205]. Thereby, as soon as enemies are not active, Cdc2/Cdc13 is becoming active. A similar mechanism is implemented in the corresponding ODE model [153] in terms of an inhomogeneous differential

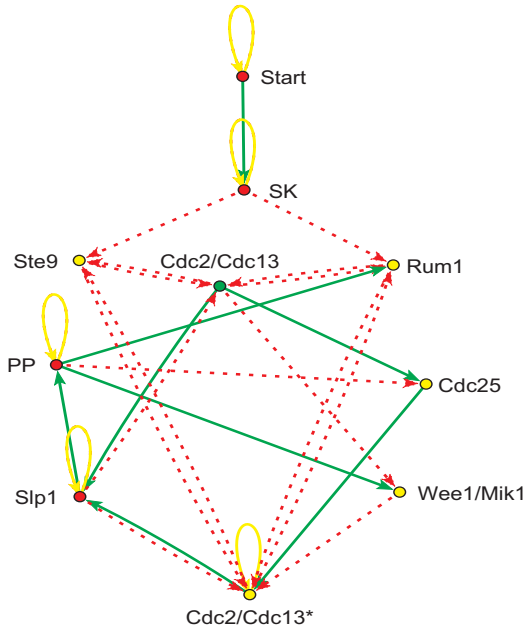

Fig. 6.1: Network model of the fission yeast cell-cycle regulation. Nodes denote threshold functions, representing the switching behavior of regulatory proteins. Arrows stand for a_{ij}, showing proteins interactions

equation for Cdc13T with a heterogeneous exciting term k_1M.

We also follow [123] by adding "self-degradation" (yellow loops) to those nodes that are not negatively regulated by others, representing the continuous degradation of proteins in the cell, which corresponds to $a_{ii} = -1$.

Nodes, that have the same function as, for example, Wee1/Mik1 and SK (Cdc2/Cig1, Cdc2/Cig2, Cdc2/Puc1) are joined together in a single node (see Figure 6.1), as it does not make a difference in the specific mathematical model dynamics considered here.

Finally let us define the initial condition of the model at the start of the simulation, which is chosen to correspond to the biological start condition, i.e. all nodes being in the OFF (inactive) state, except for the proteins Start, Ste9, Rum1, and Wee1/Mik1 [205].

6.2 Results of simulation of the Boolean model of the fission yeast cell cycle

Let us first consider the time evolution of the proteins of the dynamical model described above. We run the cell cycle model by exciting the G1 stationary state with the cell size signal ("Start" node). This initiates a sequence of network activation states of proteins that, eventually, return to the G1 stationary state. The temporal evolution of the protein states is presented in Table 6.2, where one observes a sequence of states which exactly matches the corresponding biological time sequence in the cell-cycle control network, from the excited G1 state (START) through S and G2 to the M phase and finally back to the stationary G1 state. This is a remarkable observation as it is unlikely to occur by chance due to the size of the state space.

In the next step we run the model starting from each one of the $2^{10} = 1024$ possible initial states. We find that each initial state flows into one of 13 stationary states (fixed points). The largest attractor belongs to a fixed point attracting 73% of all network states. Our first observation is that this fixed point exactly coincides with the biological G1 stationary state (see Table 6.3) of the cell. Thus, the biological target state is the dominant attractor of the network dynamics. As soon as the system reaches this state with the specific corresponding combination of active and inactive proteins, it stays there, and is likely to do so even in the presence of perturbations.

A further observation is best depicted by Figure 6.2, showing the dynamical flow of the network states, and how it converges towards the biological fixed point. In this figure, the dynamical trajectories in the state space starting from all 1024 possible initial states of the network are shown. Each network state is represented by a dot, with the arrows between them indicating the dynamical transition from one state to its temporally subsequent state. At the root of the largest attractor (tree) the G1 state is found and the blue arrows show the biological time sequence that leads to it.

Time Step	Start	SK	Cdc2 /Cdc13	Ste9	Rum1	Slp1	Cdc2 /Cdc13*	Wee1 Mik1	Cdc25	PP	Phase	comments
1	1	0	0	1	1	0	0	1	0	0	START	Cell reached critical size it starts to clock the system and activates Starter kinases
2	0	1	0	1	1	0	0	1	0	0	G1	SK are becoming active
3	0	0	0	0	0	0	0	1	0	0	G1/S	When Cdc2/Cdc13 and SK dimers switch off Rum1 and Ste9/APC, the cell passes 'Start' and DNA replication takes place, Cdc2/Cdc13 starts to accumulate
4	0	0	1	0	0	0	0	1	0	0	G2	Activity of Cdc2/Cdc13 achieves moderate level, which is enough for entering G2 phase but not mitosis, since Wee1/Mik1 inhibits the residue of Cdc2–Tyr15
5	0	0	1	0	0	0	0	0	1	0	G2	moderate activity Cdc2/Cdc13 activates Cdc25
6	0	0	1	0	0	0	1	0	1	0	G2/M	Cdc25 reverses phosphorylation, removing the inhibiting phosphate group and activating Tyr15
7	0	0	1	0	0	1	1	0	1	0	G2/M	Cdc2/Cdc13 reaches high activity level sufficient to activate Slp1/APC (Cdc2/Cdc13 and Tyr15 are both active) and cell enters mitosis
8	0	0	0	0	0	1	1	0	0	1	M	Slp1 degrades Cdc13 and activates unknown phosphase
9	0	0	0	1	1	0	1	1	0	1	M	Antagonists of Cdc2/Cdc13 are reset
10	0	0	0	1	1	0	0	1	0	0	G1	Cdc13 is degraded, Cdc2 thereby downregulated, cell reaches G1 stationary state

Tab. 6.2: Temporal evolution of protein states in the cell cycle network.

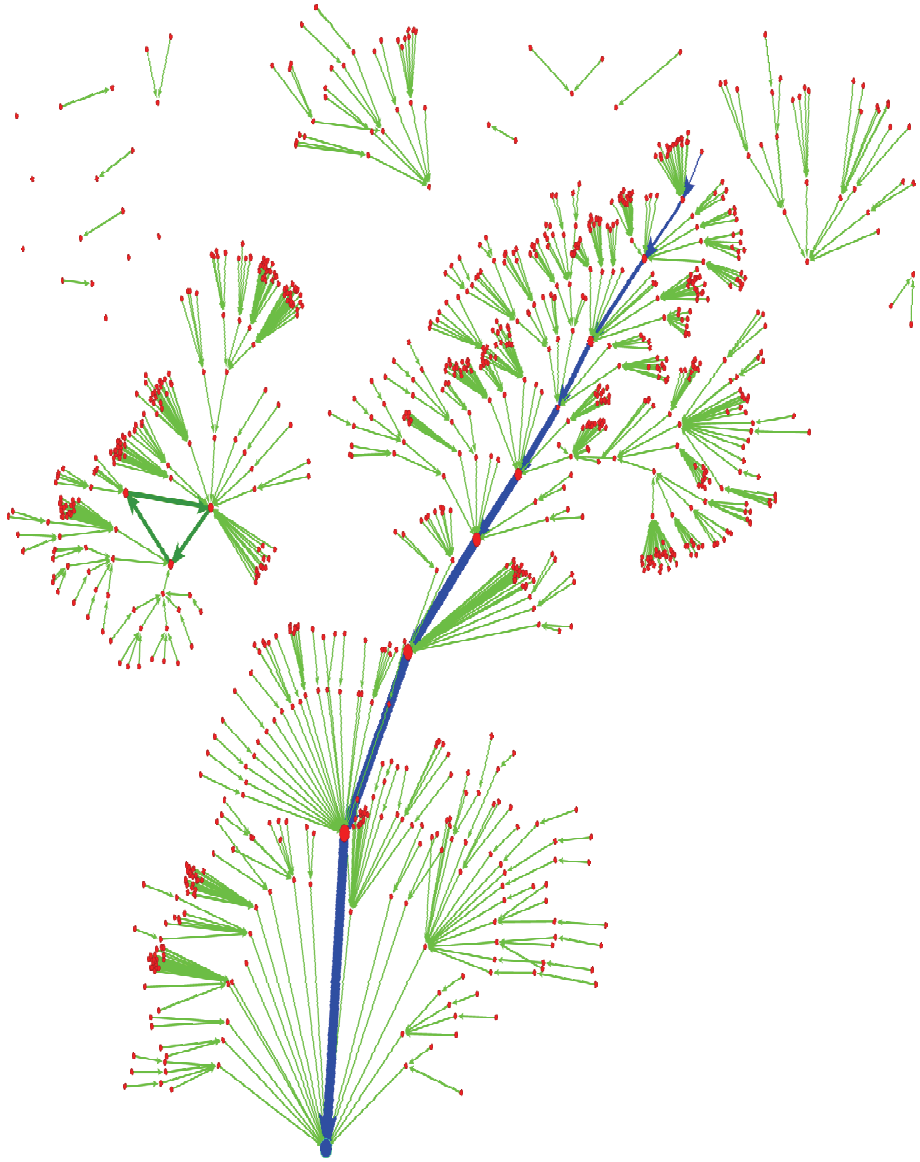

Fig. 6.2: State space of the 1024 possible network states (green circles) and their dynamical trajectories, all converging towards fixed point attractors. Each circle corresponds to one specific network state with each of the ten proteins being in one specific activation state (active/inactive). The largest attractor tree corresponds to all network states flowing to the G1 fixed point (blue node). Arrows between the network states indicate the direction of the dynamical flow from one network state to its subsequent state. The fission yeast cell-cycle sequence is shown with blue arrows.

Attractor	Basin size	Start	SK	Cdc2/ Cdc13	Ste9	Rum1	Slp1	Cdc2/ Cdc13*	Wee1/ Mik1	Cdc25	PP
1	762	0	0	0	1	1	0	1	0	0	0
2	208	0	0	0	0	0	0	0	0	1	1
		0	0	0	0	0	1	0	0	1	0
		0	0	1	1	1	0	1	1	0	0
3	18	0	0	0	0	1	0	0	1	0	0
4	18	0	0	0	1	0	0	0	1	0	0
5	2	0	0	0	1	0	0	0	0	0	0
6	2	0	0	0	1	0	0	0	0	1	0
7	2	0	0	0	1	0	0	0	1	1	0
8	2	0	0	0	0	1	0	0	0	0	0
9	2	0	0	0	0	1	0	0	0	0	0
10	2	0	0	0	0	1	0	0	0	1	0
11	2	0	0	0	1	1	0	0	0	0	0
12	2	0	0	0	1	1	0	0	0	1	0
13	2	0	0	0	1	1	0	0	1	1	0

Tab. 6.3: All attractors (fixed points and one limit cycle (attractor number 2)) of the dynamics of the network model for the fission yeast cell cycle regulation.

This attractor tree consists of 73% of all network states.

We further performed a typical testing of the models, described in 3.2.3, a robustness test by reversing the state of a single, randomly chosen node while the network proceeds through the biological sequence. This deviation from the biological pathway by the activity state of one single protein at one randomly chosen step of the cycle, the system returns to the fixed point G1 in 90 out of 100 possible cases. Thus we observe an additional robustness in the fission yeast cell-cycle network, meaning that there is an increased probability to stay in the attractor basin of the biological fixed point when perturbing states along the biological trajectory.

An immediate question about the specific network structure considered here is whether the architecture of the network has special properties as, for example, traces of being optimized by biological evolution. We compare the network dynamics to the null model of random networks with the same number of inhibiting and activating links, self-degrading and self-activating nodes and the same activation thresholds. Indeed one finds that the corresponding random networks typically have smaller attractors. The mean size of the biggest attractors is about 38% of all initial states

(averaged over 1000 random networks). This may indicate that attractor basin size of the biological attractor is optimized to provide additional dynamical robustness.

These results suggest that the research of RBN without knowing a particular topology of certain regulatory networks has a limited potential. It gives very mean picture of the process, averaging all possible different networks, whereas real biological regulatory networks can have very specific topology, which deviates from a mean significantly. Therefore, the dynamical properties of the system will be also different than in a corresponding RBN model.

Fission yeast	Rum1	Ste9	Slp1	Cdc2	Cdc13
Budding yeast	Sic1	Cdh11	Cdc20	Cdc28	Clb1-6

Tab. 6.4: Homologue proteins related to the cell cycle networks of fission yeast and budding yeast

6.3 Comparison with S. cerevisiae (budding yeast)

The two yeasts, *S. cerevisiae* and *S. pombe*, are remarkably different cells and a comparison may provide insights relevant for the understanding of higher eukaryotic organisms. As we now have discrete dynamical models for the cell cycle network of both of them at hand (this work, as well as [123]), let us discuss how they compare.

As these two organisms are closely related genetically, one might expect a large overlap also in the biochemical control machinery. On the other hand, the biology of the two is markedly different, so there have to be some differences on the biochemical level as well. As an overview, the second model is shown in Figure 6.3.

There are a number of closely related genes (see Table 6.4) between the two yeasts [60], which, however, can have vastly differing functions [204]. In fission yeast, for example, phosphatase Cdc25 is required for the G2M transition, while in the model of budding yeast [123] the corresponding homologue Mih1 is insignificant. The reason is that in the fission yeast cell cycle, Cdc25 removes an inhibitory phosphate group from the residue Tyr-15 of Cdc2, which is important for the right timing of the G2M transition. In contrast, the tyrosine residue in S. cerevisiae Cdc28 kinase (fission yeast: Cdc2) is not as critical and usually not phosphorylated. Therefore, for a model of fission yeast, Cdc25 is essential, whereas the homologue Mih1 in budding yeast is not [60]. One other example is the role of the protein Cdc13. In fission yeast it acts in a complex with Cdc2, while in the budding yeast model its functionality is represented

by two complexes Clb1,2/Cdc28 and Clb5,6/Cdc28, which exhibit some differences in interactions, as well as in timing.

Despite of the differences in many details, the general logic of both yeast cell cycles is surprisingly similar and exhibits a number of "structural homologues". For example, both exhibit a negative feedback loop similar in role: in budding yeast Clb1,2/Cdc28 activates Cdc20 which inhibits Clb1,2/Cdc28, in fission yeast Cdc2/Cdc13 activates through Cdc25 Cdc2/Cdc13*, which activates Slp1, which in turn inhibits Cdc2/Cdc13, Cdc2/Cdc13*.

The most interesting comparison is in our view on the level of the global network dynamics. From this point of view, the S. cerevisiae network is a strongly damped system, driven by external excitation. External signals are entering the network, triggering signal cascades in the network that induce the subsequent phases. In contrast, the network of S. pombe corresponds to an auto-excited system (driven by a node with self-excitation-Cdc2/Cdc13) with additional damping. Here, an external signal works as a trigger mechanism that counteracts internal damping, causing the auto-excitation to spread its activity in the system.

While these differences in the mechanics of the signaling networks are considerable, the overall dynamics is surprisingly similar. The state space picture is quite similar in both cases: one observes only a small number of attractors and just one big global attractor (with 86% resp. 73% of all initial states), which for both organisms corresponds to the stationary G1 state.

Finally, a most prominent difference between the two yeast networks is their choice in biochemical machinery: S. cerevisiae relies more on transcriptional factors while S. pombe mostly relies on post-translational regulation [182]. From the methodological point of view, we note that for this reason we were surprised to find our model for the S. pombe cell cycle network so robust against neglecting the vastly different time scales of interactions, which we expected to be the major difficulty in constructing a

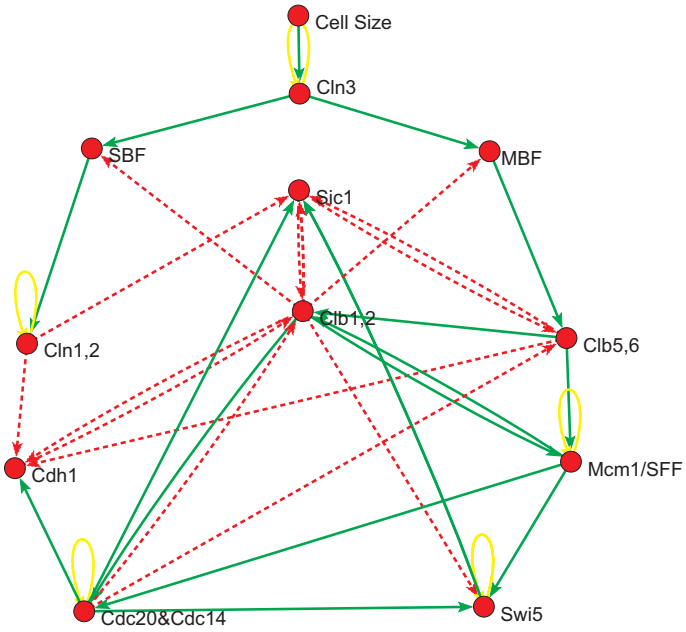

Fig. 6.3: Budding yeast cell cycle network model of [123], for comparison with our model of fission yeast. This network relies more on transcriptional regulation than the fission yeast network (note that some homologues corresponding to the latter do not have to be included here). Note also the difference in circuitry.

discrete dynamical model for S. pombe as compared to S. cerevisiae.

6.4 Discussion

We have constructed a Boolean model for the biochemical network that controls the cell cycle progression in fission yeast *S. pombe*, and found a number of interesting results. The dynamics of this network reproduces the time sequence protein activation along the biological cell cycle, solely on the basis of the connectivity graph of the network, neglecting all biochemical kinetic parameters. The dynamics of the network is characterized by a dominant attractor in the space of all possible states, with an attractor basin that attracts most of all states. The network dynamics is robust against perturbation of the biological sequence of protein activation.

Also there is an interesting result, that the second big attractor is a limit cycle. This limit cycle could be related to the Wee1-Cdc25 double mutant. These cells have

quantized cell cycles [204] as a result of an underlying oscillator that creates small amplitude oscillations in Cdc2/Cdc13 activity (with a role of Slp1 in this).

The results obtained from our model are in accordance with the existing NT ODE model of fission yeast, described in the previous chapter [204]. Let us discuss the differences between these two approaches. The *S. pombe* ODE system [205] has several steady state solutions. One can identify every such solution with the corresponding physiological stage. As it has been shown in 5.3, the growth of cell size brings the cell from one phase to another via a series of bifurcations. At the same time, other variables indicate the degree of activity of various components of the cell regulatory nodes. One observes in Fig. 5.3 [204] that the typical curves depicting this activity have almost rectangular shape. This motivates our choice of binary valued function to approximate protein concentrations in time (the complete mathematical transition between ODE and Boolean network model will be demonstrated in chapter 8). Further, the ODE-based model makes use of continuous system parameters, which we omit and replace by their signs, only. As a result, the ODE bifurcation curve then corresponds to the Boolean biological path. The main advantage of our Boolean model is that we were able to drop 47 kinetic constants that were necessary in the ODE approach and, while doing so, still reproduce the biological sequence of protein activation.

As it has been shown in 5.3 the growth of cell size brings the cell from one phase to another via a series of bifurcations. At the same time, other variables indicate the degree of activity of various components of the cell regulatory nodes. One observes in Fig. 5.3 [205] that concentrations of the major proteins rise and decrease steeply.

This fact and our further observations point at built-in dynamical robustness of the network, which may provide a mechanism for organisms to ensure functional robustness [11]. Vice versa, our study indicates that the regulatory robustness of biological chemical networks may allow for "robust" modeling approaches: Our paradigm here

is nothing but assuming that biochemical networks are functioning in a parameter-insensitive way, which motivated us to eliminate tunable parameters all together. That our model reproduces the biological sequence instantly without any further parameter tuning, confirms our assumption a posteriori. We therefore encourage further modeling experiments with the here presented, quite minimalistic approach as it may prove a quick route to predicting biologically relevant dynamical features of genetic and protein networks in the living cell.

The next chapter describes a verification of the model by testing different mutations on an extended version of the model, presented in this chapter.

7. MODELING MUTATIONS WITH A BOOLEAN NETWORK APPROACH

This chapter starts with an overview on the existing Boolean network models simulating mutations, followed with an extended version of a fission yeast cell cycle model, introduced in the previous chapter. Finally the possibilities and limitations of the Boolean network approach are discussed and compared with the differential equation method.

7.1 Introduction to models of mutations

As it was already mentioned in previous chapters, the protein-protein interactions are very complicated and the knowledge of individual properties of proteins and interactions is not able to comprehend the whole complexity. In particular, in the previous chapters 2 and 3 different experimental and theoretical methods are described, which are used for investigating the control mechanisms of protein-protein interactions. Here we want to concentrate on one particular method of model verification – reproduction of mutations. Reproduction of mutations serves verification of model validity as is of vital importance for finding the key-proteins and understanding the control mechanisms of the processes.

Different mathematical models have been developed for this purpose. In some cases, models have proven to make predictions for mutations that have been confirmed by experiments. The choice of a method for a particular network depends

on the level of organization of that network. For instance, probabilistic estimations are used for identifying single nucleotide polymorphism that can potentially effect protein function [150]; automated methods using score function are applied for point-mutations in protein structure [59]; hybrid discrete-continuum technique has been used for mathematical model for thermal growth of a tumor after mutations of oncogeneses and anti-oncogeneses [181]. The most common approach involves ordinary differential equations (ODE). This method has been used for predictions of some mutations in major for budding yeast, fission yeast and the mammalian cell cycle [34, 154, 155].

The mutations in Boolean network models are typically simulated as the insertion and deletion of nodes and links [220]. The Boolean approach has been successfully applied for modeling wild-type and mutants in a number of biological processes such as cell-fate determination during Arabidopsis thaliana flower development [55], mammalian cell-cycle [56] and the neurotransmitter signaling pathway [82]. One of the pioneering works within this approach used a Boolean gene network model for a Drosophila melongaster and successfully reproduced the knock-out mutations of transcription factors [7]. There were also some attempts to construct Boolean models of an artificial genome at the level of a nucleotide sequence and to map it into regulatory network [220]. A common feature in all these models is that logical Boolean functions, determining changes in the next time step, are separately identified for every node and use logical operators "and", "or" and "not". In spite of these successful examples, it is still not clear whether this approach is accurate enough for describing a large number of single as well as double and/or triple mutations.

The goal of this chapter is to determine how far one can get with the Boolean network approach, in particular whether it is an appropriate method for creating reliable models that capture the properties of the system including mutations. We also aim to answer the question whether it is possible to formulate one general logical

function for all nodes without creating individual rules for every node. For this study, we further use *Schizosacharomyces Pombe* (fission yeast) for which a lot of data is available [205, 206]. NT models described in the chapter 5 are able to reproduce not only the evolution of key-protein concentrations during the cell cycle for a wild-type but also for a number of temperature-sensitive, loss-of-function and overexpressing mutants [195]. A very interesting question is whether it is possible to build a reliable Boolean model that is free from kinetic constants, which is the main challenge in ODE method, yet describes the progression through the cell cycle and accounts for mutations?

In the previous chapter, we built the Boolean model of the fission yeast cell that is solely based on known interaction topology, does not require knowing the kinetic constants [43] and reproduces the known activity sequence of regulatory proteins in the cell cycle for a wild type. In this chapter we show that it is possible to extend this model that it also reproduces a large number of mutants. We determine what kind of mutations can and cannot be modeled within the Boolean approach. The chapter is organized as follows. In the next section we first describe our model based on known biochemical reactions, which is followed by the results for a wild type and a number of known mutant cells. In Sec. 7.4 we discuss the differences and similarities in results for wild and mutant cells in the Boolean network and ODE models.

7.2 Results

7.2.1 Extended model of the fission yeast cell cycle

The model which is described in a previous chapter was extended for being able to reproduce a large number of mutations for fission yeast cell cycle. In particular, the previous model does not distinguish between two possible states of Cdc2 - Cdc2 phosphorylated or not, reducing these two states to two possible states of Cdc2/Cdc13

complex. Here we recognize phosphorylated and unphosphorylated residue Tyr15 described above which allows us to improve model [43] and better grasp the underlying mechanisms of the process. The concentration of Cdc2 does not change during the cell cycle, however it can exist in two states: (1) phosphorylated on residue Tyr-15 or (2) not phosphorylated. The phosphorylation of Tyr-15 reduces the activity of Cdc2. For this reason, we add an extra node Cdc2_Tyr15 that describes the phosphorylation state of Cdc2. This node is ON if phosphorylation is removed and is OFF otherwise. The activation of Cdc2_Tyr-15 together with Cdc2/Cdc13 is crucial for G2-M transition, whereas the activation of only Cdc2/Cdc13 without Cdc2_Tyr-15 corresponds to G2 phase [154, 205].

Also in model [43] all Start Kinases (SK) are represented by one node - SK. Here we extend our model by distinguishing between three start kinases – $Cig1/Cdc2$, $Cig2/Cdc2$, $Puc1/Cdc2$ and model them as three different nodes which gives a possibility to reproduce more mutations.

We give a summary of the interactions between key-regulators of the fission yeast cell cycle network in Table 7.1. Fig. 7.1. visualizes Table 7.1, where protein and complexes are represented by nodes of network and all types of reactions are reduced to activation/inhibition and are shown by green/red links correspondingly.

All nodes are updated parallel in discrete steps according to the rule (6.1) introduced in the previous chapter keeping the same θ for the same nodes.

7.3 Results of Boolean simulation of the fission yeast cell cycle

7.3.1 Wild type

First repeat the same tests for improved version of the model as it was done for the original version of the Boolean network model introduced in the previous chapter. We run our model starting from biological conditions which are chosen in correspondence

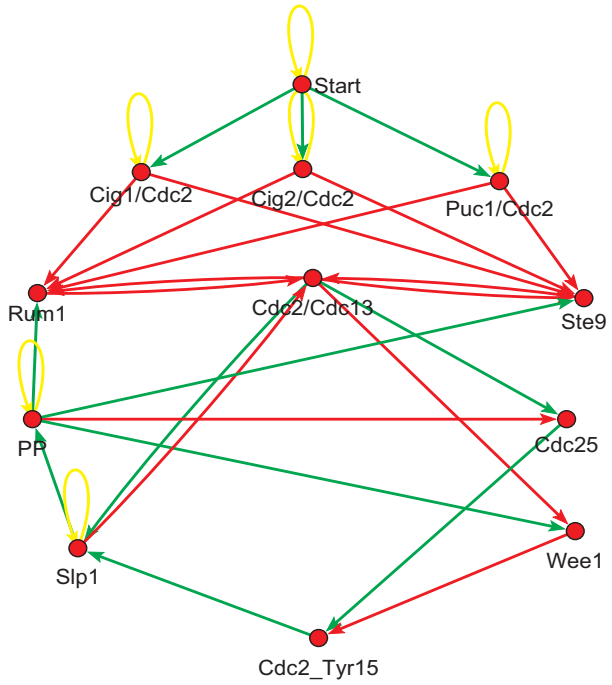

Fig. 7.1: Network model of the fission yeast cell-cycle regulation.

with biological start conditions, i.e. all nodes are in the OFF (inactive) state, except for the nodes Start, Ste9, Rum1, and Wee1 [205]. The initiated sequence of states of the network, which is the sequence of ON/OFF states of the nodes, reproduces the biological time sequence of proteins activation during wild-type cell cycle phases G1 - S - G2 - M - G1. The Table 7.2 and Fig. 7.2 confirm that. The last time step corresponds to G1 stationary state, where the activity of all nodes is the same as at first time step, except for the Start node.

In the next step we run the model starting from each one of the $2^{12} = 4096$ possible initial states. We find that each initial state flows into one of 15 stationary states (fixed points). The largest attractor belongs to a fixed point attracting 77% of all network states. Our first observation is that this fixed point exactly coincides with the biological G1 stationary state (see Fig. 7.4) of the cell. Thus, the biological target state is the dominant attractor of the network dynamics. As soon as the system reaches this state with the specific corresponding combination of active and inactive

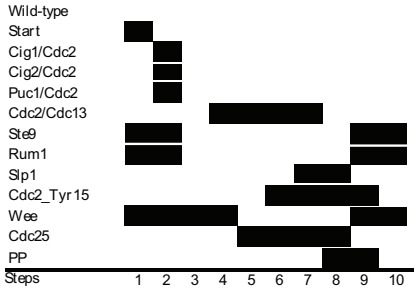

Fig. 7.2: The temporal evolution of protein state of wild-type cell-cycle

proteins, it stays there, and is likely to do so even in the presence of perturbations.

7.3.2 Mutations

A consistent model should be able to describe not only the wild-type but also the mutants of the fission yeast cell cycle. There are at least three possible types of mutations: temperature-sensitive, loss-of-function and overexpressing mutants. The first type corresponds to reduced activity, the second type to zero-activity and the third type to an overproduced activity of a protein. For modeling the temperature-sensitive mutants in [191, 154, 205] the appropriate kinetic constants are reduced by 10%, for loss-of-function mutants these constants are set to zero, and for overexpressing mutants they are increased by a factor of several.

In terms of the Boolean approach one cannot model temperature-sensitive mutations when the activity of proteins changes slightly. For this reason we model mostly loss-of-function and overexpressing mutations. In order to model loss-of-function $\triangle$ mutations, we delete from the network the mutation node and run the updated model. Below we describe the dynamical properties and biological explanations of modeled mutations. Table 7.3 summarizes the properties of mutations.

Parent node	Daughter node	Rule of activation (comments)	Rule of inhibition (comments)
Start node	Kinases (SK): Cdc2/Cig1, Cdc2/Cig2, Cdc2/Puc1	Start node works as an indicator of mass of the cell and activates Starter Kinases (SK): Cdc2/Cig1, Cdc2/Cig2, Cdc2/Puc1, +1 [191].	
SK	Ste9, Rum1		Phosphorylate, thereby inactivate, -1 [191, 205]
Cdc2/Cdc13	Cdc25	Cdc25 is phosphorylated thereby activated, +1 [191].	
Wee1, Mik1	Tyr15		Phosphorylate, inactivating, -1 [191]
Rum1	Cdc2/Cdc13		Binds and inhibits activity, -1 Cdc2/Cdc13 [191].
Cdc2/Cdc13	Rum1		Phosphorylates and thereby targets Rum1 for degradation. -1 [191, 205]
Ste9	Cdc2/Cdc13		Labels Cdc13 for degradation [205, 191], -1.
Tyr15, Cdc2/Cdc13	Slp1	Highly activated Cdc2/Cdc13 activates Slp1, Tyr15 has to be active, too [154, 191]+1.	
Slp1	Cdc2/Cdc13		Promotes degradation of Cdc13, thereby the activity of Cdc2/Cdc13 drops -1 [191]
Slp1	PP	Activates, +1 [191]	
PP(Unknown phosphatase)	Ste9, Rum1, Wee1, Mik1	Activates Rum1, Ste9, and the tyrosine-modifying enzymes (Wee1, Mik1, [191], +1	
Cdc25	Tyr15	Cdc25 reverses phosphorylation of Cdc2, thereby Tyr15 becomes active, +1 [191, 154]	
Cdc2/Cdc13	Ste9		inhibits -1 [154]
PP	Cdc25		inhibits -1[191]
Cdc2/Cdc13	Wee1, Mik1		inhibits -1 [154]

Tab. 7.1: The rules of interaction of the main elements involved in the fission yeast cell cycle regulation.

Time	Start	Cig1 /Cdc2	Cig2 /Cdc2	Puc1 /Cdc2	Cdc2 /Cdc13	Ste9	Rum1	Slp1	Tyr15	Wee1	Cdc25	PP	Phase	comments
1	1	0	0	0	0	1	1	0	0	1	0	0	START	G1 START G1 Cdc2/Cdc13 dimers are inhibited, antagonists are active.
2	0	1	1	1	0	1	1	0	0	1	0	0	G1	SK are becoming active
3	0	0	0	0	0	0	0	0	0	1	0	0	G1/S	G1/S When Cdc2/Cdc13 and SK dimers switch off Rum1, and Ste9/APC, the cell passes 'Start' and DNA replication takes place, so Cdc2/Cdc13 starts to accumulate its activity
4	0	0	0	0	1	0	0	0	0	1	0	0	G2	Activity of Cdc2/Cdc13 achieves moderate level, which is enough for entering G2 phase, but not mitosis, since Wee1/Mik1 inhibits residue of Cdc2-Tyr15, that doesn't allow total activation
5	0	0	0	0	1	0	0	0	0	0	1	0	G2	With moderate activity Cdc2/Cdc13 can activate Cdc25
6	0	0	0	0	1	0	0	0	1	0	1	0	G2/M	Cdc25 reverses phosphorylation, removing inhibiting phosphate group and making residue of Cdc2-Tyr15 active
7	0		0	0	1	0	0	1	1	0	1	0	G2/M	Cdc2/Cdc13 reaches high activity which is enough for activating Slp1/APC (Cdc2/Cdc13 and Tyr15 are both active) and cell enters mitosis
8	0	0	0	0	0	0	0	1	1	0	1	1	M	Slp1 degradates Cdc13 and activates unknown phosphase
9	0	0	0	0	0	1	1	0	1	1	0	1	M	Antagonists of Cdc2/Cdc13 are resetted
10	0	0	0	0	0	1	1	0	0	1	0	0	G1	Cdc2 loses it's activity because, because Cdc13 is degraded, cell achives G1 stationary state

Tab. 7.2: Temporal evolution of protein states for the cell-cycle network, wild type.

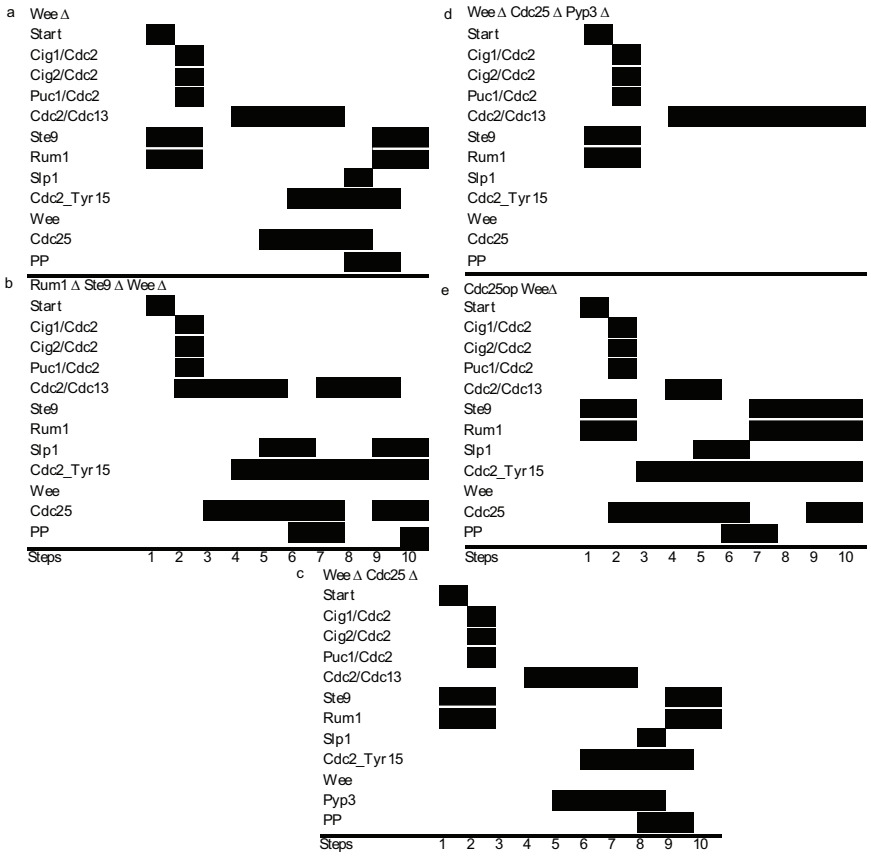

Fig. 7.3: The temporal evolution of protein state of Wee and Cdc25 mutant cells. The black/white color responds to active/inactive state of a protein correspondingly.

Wee△ and Cdc25△ mutants

The duration of S and G2 phases are controlled by down-regulation of Wee by Cdc2/Cdc13. If Wee is absent (Wee△), then the cell enters mitosis with a smaller size, but it stays viable [155]. The modeling of Wee△ confirms this. The temporal evolution of protein states stays the same as in wild-type. The system has one fixed point which corresponds to the G1 stable state (Fig. 7.3 a)

However, if some other antagonists of Cdc2/Cdc13 are also mutated, e.g. Rum1△ - Wee△ or Ste9△Wee△, then the cells divide too fast and do not have enough time

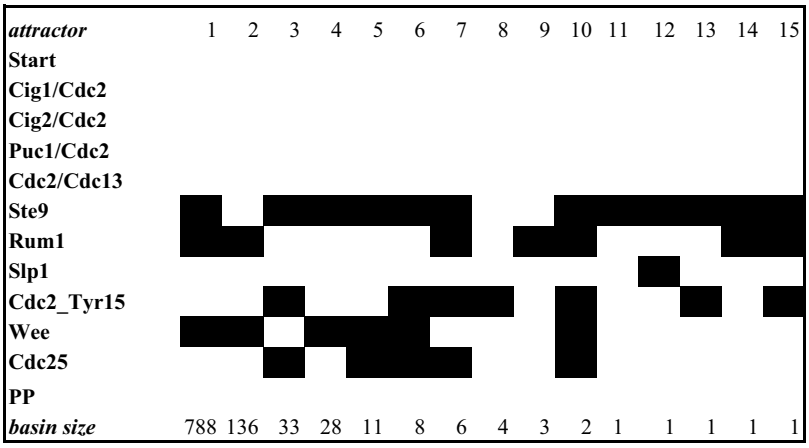

Fig. 7.4: All attractors of the dynamics of the network model for the wild-type fission yeast cell cycle regulation

to grow [191]. With every division cells get smaller and smaller until they die. In our model Starter kinases – Cig1/Cdc2, Cig2/Cdc2 and Puc1/Cdc2 are not influenced by *Rum*1 and *Ste*9 for simplicity. In fact Cig2 is partly inhibited by Rum1 and possibly by Ste9 [154, 191]. For this reason one cannot separate Wee$\triangle$ and Rum1$\triangle$Wee$\triangle$, Ste9$\triangle$Wee$\triangle$ mutations. However, our model reproduces the triple mutation Rum1$\triangle$Ste9$\triangle$Wee$\triangle$. In this case the system shows oscillations and is not viable. The cell divides uncontrollably and the temporal evolution of protein states is shown in Fig.7. 3 b – step 10 repeats step 6, that is the system goes periodically through the same sequence of states.

In order to understand the Wee$\triangle$Cdc25$\triangle$ mutation, one has to take into account that Cdc25 has a back-up enzyme, called Pyp3. Pyp3 is a tyrosine-phosphate with a much lower activity, which means that dephosphorylation of Cdc2 is non zero, when Pyp3 is present. Therefore one can model Wee$\triangle$Cdc25$\triangle$ mutation as follows: Node Wee is deleted and the weight of the link connecting Cdc25 to Cdc2_Tyr15 is set to 0.5 instead of the usual 1. This results in a vital mutation, when the cell goes through all phases, and the temporal evolution of proteins is the same as for a wild cell, except

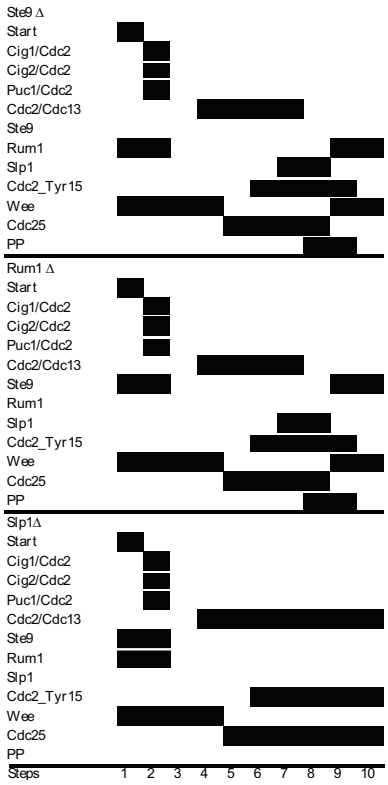

Fig. 7.5: The temporal evolution of protein state of Ste9, Rum1 and Slp1 mutant cells. The black/white color responds to active/inactive state of a protein correspondingly.

the Wee, which is for this mutation OFF (Fig 7. 3 c). This is confirmed by experimental data [191]. The removal of the nodes Cdc25 and Wee corresponds to a triple mutant Wee$\triangle$Cdc25$\triangle$Pyp3$\triangle$, when Tyr15 stays phosphorylated. This mutation is not viable. The cell cannot enter mitosis, since Tyr15 stays phosphorylated, thereby preventing Cdc2/Cdc13 to reach high activity. Our model reproduces this, one can see in Fig. 7. 3 d that Cdc2_Tyr15 stays inactive and the cell cannot enter mitosis.

Overexpression of proteins can be modeled as following. Overexpression means that the activity is significantly increased. In the frames of our model overexpression is interpreted in a such a way that overexpressed protein has a constant positive input, which corresponds to a negative *theta* in (6.1). Here and further we choose *theta* = -0.5 for all overexpressed mutants. Therefore for modeling Wee^{ts} $Cdc25^{op}$ since there

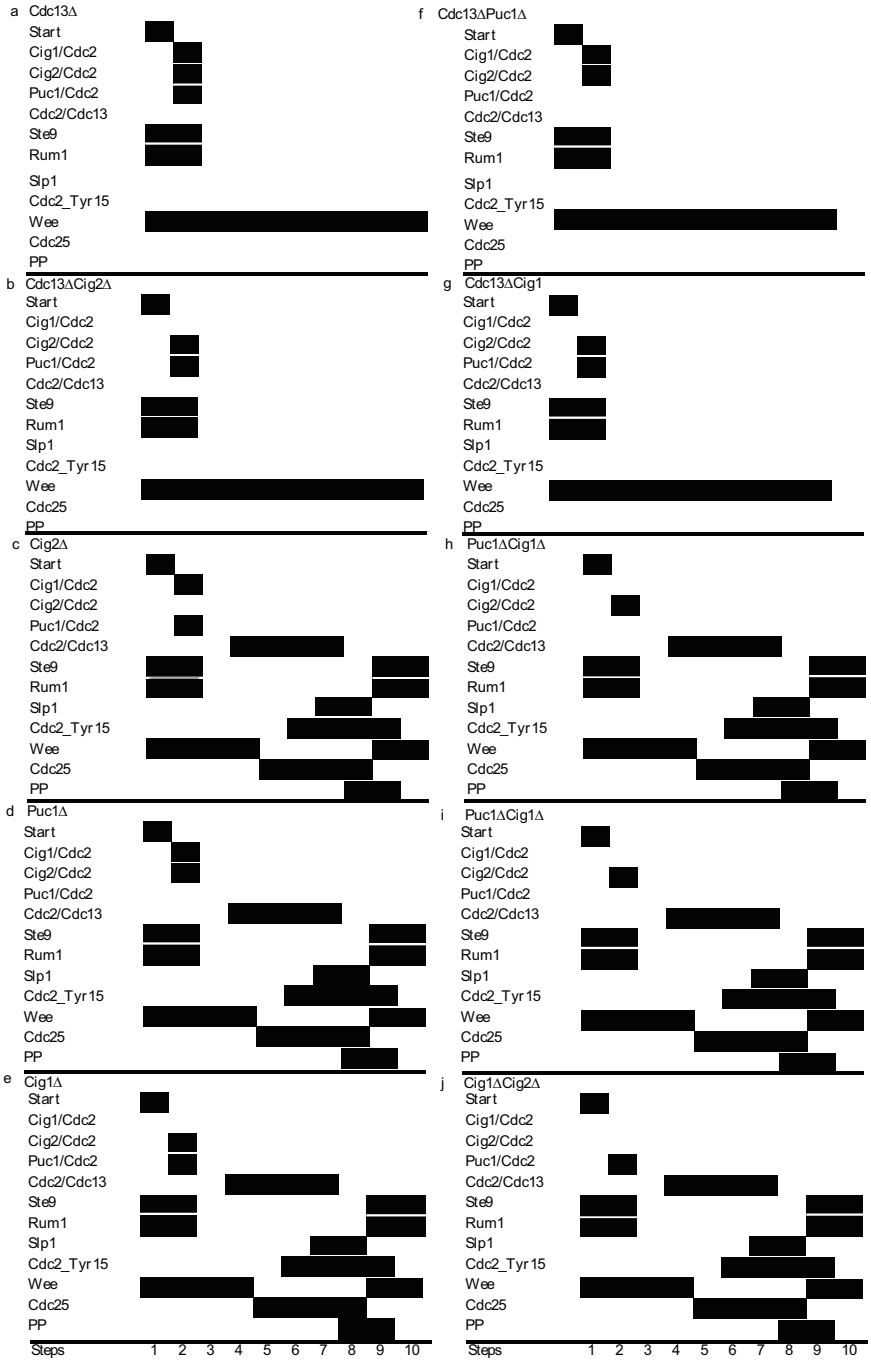

Fig. 7.6: The temporal evolution of protein state of Start kinases mutant cells. Part 1. The black/white color responds to active/inactive state of a protein correspondingly.

is no way to distinguish between reduced activity and no-activity, mutation Wee^{ts} was substituted with Wee$\triangle$ and the threshold of activation was changed to -0.5 for Cdc25. In Fig. 7. 3e one sees that mitosis happens very quickly without having appropriate G2 phase, which means that mitosis is initiated before the replication of DNA was completed. In case of overexpression of Wee the cell remains viable (Fig 7. 9 b). Experimental observations confirm these results [166].

Mutations of Cdc2/Cdc13 antagonists: Ste9$\triangle$, Rum1$\triangle$, Slp1$\triangle$ mutants

Fission yeast survives in the absence of Ste9 or Rum1 [110] and grows normally. Our observations of temporal protein evolution confirm this fact (Fig. 7.5 a, b). The system has one fixed point G1 that is reached after the evolution through all G1-S-G2-M phases. However, the absence of the other Cdc2/Cdc13 antagonist – Slp1 – has a lethal effect. The recent studies [111] show that Slp1$\triangle$ is a lethal mutation that prevents the mitosis. The dynamical behavior of the model for Slp1$\triangle$ shows that the system reaches a fixed point, which corresponds to G2 late phase, right before entering mitosis. The evolution of the proteins first coincides with wild-type, but then stays freezing at step 6 (Fig. 7. 5 c).

Mutations of cyclins: Cig1$\triangle$, Cig2$\triangle$, Puc1$\triangle$, Cdc2$\triangle$ and cyclin-dependent kinase Cdc13$\triangle$

The only cyclin which makes fission yeast to die is Cdc13. The presence of Cdc13 is essential for normal progression through the cell cycle [191]. In the absence of Cdc13 the cells elongate abnormally and cannot enter mitosis. Our simulation confirm this (Fig. 7.8 a): The Start Kinases Cig1$\triangle Cdc2$, Cig2$\triangle$Cdc2, Puc1$\triangle$Cdc2 switch off the Cdc2/Cdc13 antagonists during G1-S phase, but in the absence of Cdc13 the cell cycle cannot evolve further. The system remains on the fourth step of wild-type cell cycle evolution (Fig. 7.6 a,b,f,g).

Start Kinase Cig1/Cdc2, Cig2/Cdc2 and Puc1/Cdc2 are responsible for deactivation of Cdc2/Cdc13 antagonists. Mutations of cyclins of Start Kinase influence only the duration of the G1 phase, which becomes longer, when they are mutated. Thereby mutants Cig1△, Cig2△, Puc1△ as well as their double mutants and triple mutants Cig1△Cig2△, Cig1△Puc1△, Cig2△Puc1△, Cig1△Cig2△Puc1△ are viable. Due to simplifications of the Start kinase interactions we made, our model is able to reproduce only single and double mutations and not triple mutations (Fig.7.6 b, c, d, e, f, g, h, i, j). Due to the fact that the time in a Boolean model is discrete, one cannot distinguish wild-type and Start Kinase mutants. The temporal evolution is similar to the wild-type. Double mutations and triple mutations Cig1△Wee^{ts}, Cig2△Wee^{ts}, Cig1△Cig2△Wee^{ts}, Puc1△ Wee^{ts}, Cig2△ - Rum1△, Ste9△Cig1△, Ste9△Puc1△, Cig1△Rum1△, Wee△Cdc2△, Wee△Cig2△ Cig1△, Ste9△Cig2△, Cig2△Rum1△, Puc1△Rum1△, Wee△Cig1△, Wee△Puc1△ are also viable (Fig 7.7 a-j).

Overexpressions of Cdc13 and Cdc2 are non-lethal, that is also proven by our model: The cell evolves through all phases as in a wild-type. The knock-out mutations (Cdc13△, Cdc2△) bring the cell to the lethal mutation and block it in G1 and G2 (Fig. 7.9).

7.4 Discussion

The improved Boolean model of the fission yeast cell cycle is constructed based on key-protein interactions documented experimentally. The discrete model reproduces the biological pathway during G1-S-G2-M phases for wild-type and for a large number of single, double and triple loss-of-functions and overexpressing mutants. The dynamical properties of the model indicate that the wild-type cell network has a dominant attractor in a state space, which coincides with biological G1 stationary state. Therefore, it is unlikely that the process will deviate from the initial one and

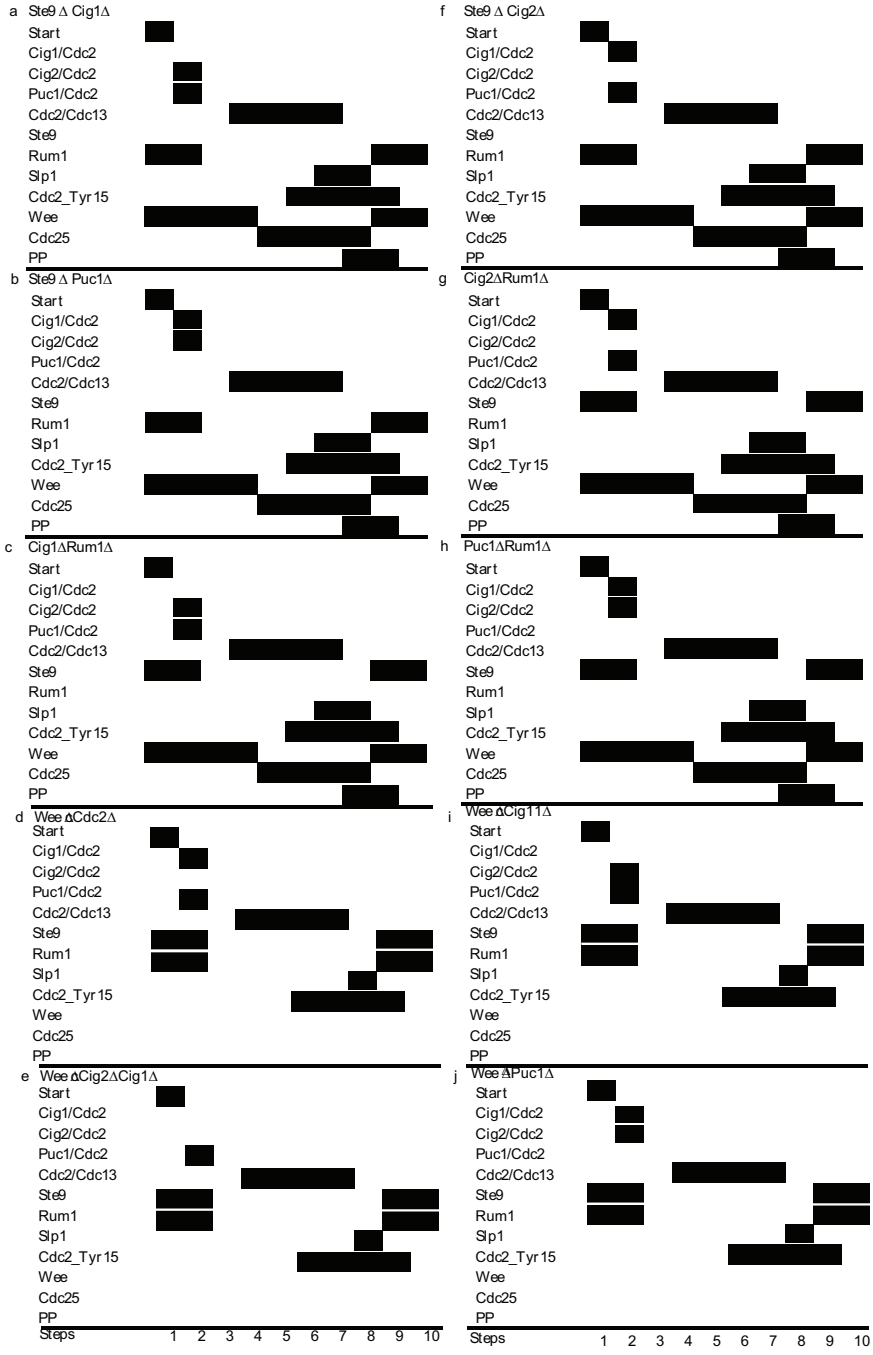

Fig. 7.7: The temporal evolution of protein state of Start kinases mutant cells. Part 2. The black/white colo responds to active/inactive state of a protein correspondingly.

Strain	Deleted node	Dynamical properties	Comment
WT	none	f. p. in G1	viable
Wee△	Wee	f.p. in G1	viable
Cdc25△	Cdc25	f.p. in G2	blocked in G2
Wee△ Cdc25△	Wee; Cdc25 is substituted with a node Pyp3 which has the same links except the strength of the link from Pyp3 to Wee1, which is substituted by -0.5 instead of previous -1	f. p. in G1	viable
Rum1△	Rum1	f.p. in G1	viable
Ste9△	Ste9	f. p. in G1	viable
Cig1△	Cdc2/Cig1	f. p. in G1	viable
Cig2△	Cdc2/Cig2	f. p. in G1	viable
Puc1△	Cdc2/Puc1	f. p. in G1	viable
Cig1△Cig2△	Cdc2/Cig1 and Cdc2/Cig2	f. p. in G1	viable
Puc1△Cig2△	Cdc2/Puc1 and Cdc2/Cig2	f. p. in G1	viable
Puc1△Cig1△	Puc1 and Cig1	f. p. in G1	viable
Cdc13△	Cdc13/Cdc2	f. p. in S	blocked between S and G2
Slp1△	Slp1	f. p. between G2 and M	blocked before M
Cig1△Rum1△	Cdc2/Cig1 and Rum1	f. p. in G1	viable
Cig2△Ste9△	Cdc2/Cig2 and Ste9	f. p. in G1	viable
Ste9△Rum1△ Wee^{ts}	Ste9, Rum1 and Wee	limit cycle, oscillations	not viable, cell does not stop to divide
Cdc13△Cig1△	Cdc13 and Cdc2/Cig1	f. p. in S	blocked between S and G2
Cdc13△ Cig2△	Cdc13 and Cdc2/Cig2	f. p. in S	blocked between S and G2
Wee△Cdc25△Pyp3△	Wee and Cdc25	f. p. in G2	blocked in G2
$Cdc25^{op}$ Wee^{ts}	Wee and θ is changed to -0.5	f. p. in G1 but the transition to mitosis is too fast without appropriate G2 phase	lethal
Cig1△Wee^{ts}	Cig1, Wee	f. p. in G1	viable
Cig2△Wee^{ts}	Cig2, Wee	f. p. in G1	viable
Cig1△Cig2△Wee^{ts}	Cig1, Cig2, Wee	f. p. in G1	viable
Puc1△Wee^{ts}	Puc1, Wee	f. p. in G1	viable
Cdc2△	Cdc2/Cdc13	f. p. in S	lethal
$Cdc13^{op}$	θ is changed to -1.5 from -1	f. p. in G1	viable
$Cdc2^{op}$	θ is changed to -1.5 from -1	f. p. in G1	viable
Wee^{op}	θ is changed to -0.5 from 0	f. p. in G1	viable

Tab. 7.3: Mutations of the fission yeast cell-cycle (f.p. denotes fixed point).

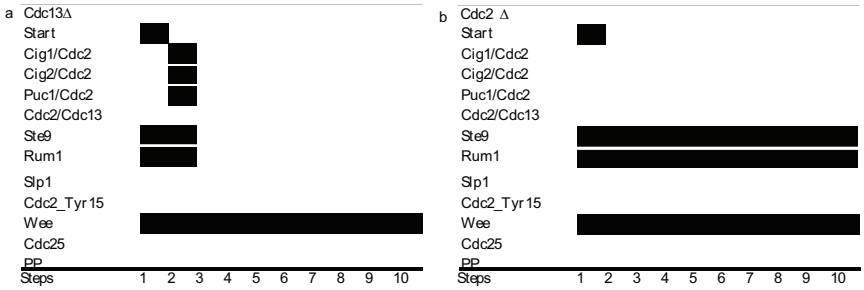

Fig. 7.8: The temporal evolution of protein state of Cdc13 and Cdc2 mutant cells. The black/white color responds to active/inactive state of a protein correspondingly.

most of mutants are viable. The different types of mutations were modeled as the following. The loss-of-function mutations were implemented by deleting the corresponding node (nodes in case of double and triple mutants correspondingly). All loss-of-function mutations were reproduced in the frames of the model except a small number. These are not reconstituted because of the dramatic simplifications that were made on the interactions between some proteins. For overexpressed mutations the additional constant positive input to activation rule was added. The reproduction of some overexpressed mutants covers not all known mutants because of the limitation of different activation states to 0/1 state in a Boolean model.

In spite of the dramatic simplification of different states of activation of proteins as well as interactions were made, the model reproduces 30 mutants, which is 70% of known-mutations in frames of involved proteins. The rest (Wee^{ts}, $Cdc25^{ts}$, $Wee^{ts}Cdc25^{ts}$, $Cdc25^{op}$, Rum1ΔWee^{ts}, Ste9ΔWee^{ts}) is not reproducible because of the discrete assumptions, i.e. one can not model temperature-sensitive (reduced 10% activity) mutants. One cannot reproduce WeeΔRum1Δ since the cell divides too fast that it does not have time to grow, which is impossible to see with discrete Boolean steps. The mutations Ste9ΔRum1Δ and Cig1ΔCig2ΔPuc1Δ are not reproducible because of the particular simplifications about interaction in the model.

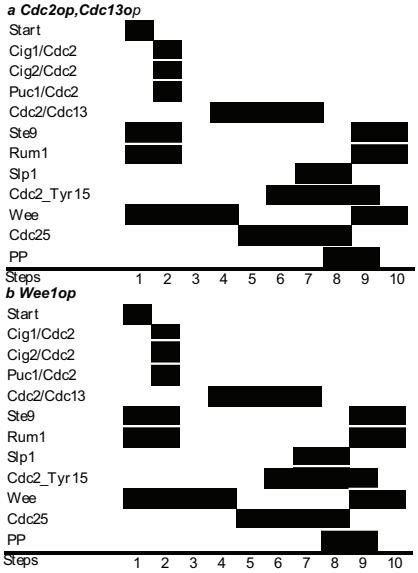

Fig. 7.9: The temporal evolution of protein state of overexpressed Cdc2 and Cdc13 mutant cells. The black/white color responds to active/inactive state of a protein correspondingly.

On the other hand the model reproduces Cig1Δ, Puc1ΔCig2Δ, Slp1Δ, Cig2ΔSte9Δ, Cdc13ΔCig1Δ, Cdc13ΔCig2Δ, Cig1ΔWeeΔ, Cig2ΔWeeΔ, Puc1ΔWeeΔ which were not modeled before. Thus, the model covers the main mechanisms of the process successfully and allows to make predictions on the modifications such as mutants that underlie changes in the phenotype.

It is interesting to compare the Boolean model with existing ODE models [151, 153, 154, 155, 191, 204, 206] for the fission yeast cell cycle. First, as it was already mentioned in chapter 5, there is no general the ODE model for the fission yeast cell cycle that would cover all details of the process simultaneously and every of ODE models is focused on this or that aspect of the process. The existing ODE models were tested for up to 22 mutations [195]. Going through all versions of models many similarities between ODE models and our Boolean model can be found. First, starting with initial conditions as in [151, 153, 154, 155, 191, 204, 206], the system evolves

through the same sequence of states. The second evidence is the robustness to the initial conditions: Our Boolean model has a dominant attractor, attracting most of the trajectories starting from different initial conditions [151, 153, 154, 155, 191, 204, 206]. The third evidence is the similarities in dynamical properties of mutations. In particular, the following mutations – Rum1△, Ste9△, Wee△, Cig1△, Cig2△, Puc1△, Cig1△Cig1△, Puc1△Cig1△, Cig1△Rum1△, Cig2△Ste9△, Wee△Cdc25△ appear to be viable in both approaches [151, 153, 154, 155, 191, 204], which is confirmed by experimental data. Non-viable mutations, such as Cdc13△, Cdc13△Cig1△, Cdc13△Cig2△, Wee△Cdc25△Pyp3△ are blocked in the same phases. It is important to mention one more similarity: Ste9△Rum1△Wee^{ts} in ODE [195] is not viable since cells are considered to be too small to be viable. In Boolean model this triple mutation results in a limit cycle. This cycle corresponds to a situation in which a cell passes through phases too quickly without waiting for a cell mass signal. Moreover, our Boolean model successfully reproduces a number of mutations mentioned above which were not modeled with the ODE approach. Thus, the current Boolean model reproduces results of ODE models, except temperature-sensitive and some overexpressive mutations. It also shows similar dynamical properties – robustness to initial conditions and to most of mutations, except the exact timing of the process.

The evident simplicity of the current model as compared to the known ODE models [151, 153, 154, 155, 191, 204, 206] is that one needs less information about biochemical reactions. In particular, it is only necessary to know whether a protein activates or inhibits another one, without additional chemical details, such as precise concentrations, difficult or non-obtainable kinetic constants that are needed by the ODE. On the other hand there are some limitations of the Boolean model. The Boolean models are not able to reproduce the exact timing but only a sequence of states due to its discrete nature. In these models reduced and absent of activity of some protein cannot be distinguished due to only two possible ON/OFF discrete

states.

The only opportunity in this case could be to try to model such kind of mutations by changing the interaction strength, as we did in case of Cdc25△. The activity of this protein couldn't be reduced to 0 in the presence of backup phosphate Pyp, where activity is much lower. Thus, one can easily model loss-of-function mutations whereas the temperature-sensitive and overexpressing mutants could appear out of reach and for these mutations more detailed models, such as ODE are needed.

7.4.1 Conclusion

Here we prove that a Boolean approach can be successfully used for modeling loss-of-function mutations and a number of overexpressive mutants (all together 30 mutants) and it is not appropriate for modeling temperature-sensitive mutations, since for last ones the more refined approach such as ODE is needed. On the other hand Boolean networks easily cover different aspects of biological process in one model with no need of building a series of models fitting kinetic constants and concentrations as in ODE models. The possible development of Boolean models that are capable to distinguish between different levels of concentration includes introduction of different discrete levels of states, distinguishing not only between 0 or 1, but 0, 1, 2 etc, as it was proposed by [198]. Another option is to represent one node with different levels of activation with two nodes or more nodes. This second approach has been implemented in the current model. The ability to model fission yeast with Boolean network method can be also interpreted as that the nature of this process is to some certain degree discrete. It also supports recent research [25] that some molecular networks are so robustly designed that timing is not a critical factor, and one can drop the requirement of accurate reproduction of time and reconstruct just a sequence of states.

Qualitative discrete modeling has been successfully applied for gene/protein regulatory networks for building predictive models in particular, modeling mutants [7, 55, 56, 82]. However, the current model is the first one that reproduces overexpressed mutations and such a huge number of double and triple mutations. The astonishing fact is that in order to grasp the comprehensive dynamical behavior we use very simple threshold rules of activation compare to complex logical rules that were used in other Boolean models. Therefore, qualitative data may be sufficient to comprehend the controlling mechanism of the system. Such a network can play a role of a starting point for further spatio-temporal dynamical models.

Qualitative discrete modeling has been successfully applied for gene/protein regulatory networks for building predictive models in particular, modeling mutants [7, 55, 56, 82]. However, the current model is the first one that reproduces overexpressed mutations and such a large number of double and triple mutations. The astonishing fact is that in order to grasp the comprehensive dynamical behavior we use very simple threshold rules of activation compared to complex logical rules that were implemented for other Boolean models. Therefore, qualitative data may be sufficient to comprehend the controlling mechanism of the system. Such a network is a starting point for further spatio-temporal dynamical models.

Summarizing, Boolean network models can be used as a rather good first approximation for modeling biological processes. This approach is able to catch the basic dynamical properties of a process and to understand the main mechanisms. One needs less information about biochemical reactions and concentrations of components for building such models. Further, Boolean models do not demand kinetic constants that cause problems in an ODE approach. As soon as one comprehends the main principles of the process, it is much easier to build more complicated and detailed models, such as ODE, in cases when more precise analysis is needed.

8. THE TRANSITION FROM DIFFERENTIAL EQUATIONS TO BOOLEAN NETWORKS

In chapters 3, 4, 5 the Boolean network and the ODE approach has been discussed constantly. In chapter 6 and 7 we compared our Boolean network model with the ODE model of the fission yeast cell cycle. The aim of this chapter is to put differential equation and Boolean network approach in correspondence to each other. We use the fission yeast cell cycle as an example system. We show here that a Boolean network model can be formulated as a specific coarse-grained limit of the more detailed differential equations model for this system. This demonstrates the mathematical foundation on which Boolean networks can be applied to biological regulatory networks in a controlled way.

8.1 Modeling biological processes with ODE and Boolean networks models

In chapter 6 and 7 we showed that our Boolean network model of the fission yeast cell cycle shares a lot of features with the ODE model. Naturally a question arises, whether these similarities are by chance or there is a mathematical foundation for it. As it has been already described in 3.1.1 and 3.2.2, the two diverse methods (ODE and Boolean networks) are both based on the same "wiring" diagram of interactions between the components, however, use much different amounts of information about these interactions. Thus, let us investigate how these two methods are related to each

other.

A first correspondence between Boolean network and ordinary differential equations has been drawn by Glass and collaborators [69, 71] who explored the relationship between a class of non-linear equations representing biochemical control networks and homologous switching networks. They argued that such a correspondence can be achieved with the following requirements: 1) Rates of reactions are described by monotonic sigmoidal functions having distinct upper and lower asymptotes. 2) The parameters must be defined to match the upper or lower asymptote. 3) The target control function must correspond to the maximal or basal rate of biochemical processes. They subsequently demonstrated that there is a large variety of such functions as, e.g., the Heaviside function, the error function, or the Hill function defined for positive arguments.

This leads to a mapping between asymptotical solutions of the ODE system and the Boolean system, while omitting the exact way of transitions between dynamical states.

Chapter 8 is organized as follows. In the next subsection we show the passage from the ODE system of algebraic differential equations for the fission yeast cell cycle, to the limit of the corresponding Boolean model which we construct. Here also the difficulties that one can meet working with Boolean approaches are discussed. After that we explore the dynamics of the derived Boolean model of the fission yeast cell-cycle. Finally, in the discussion section the properties of the obtained system are recapitulated and the Boolean and ODE approaches are compared.

8.2 Boolean variables as stationary states

The passage from a differential equations model to a Boolean model requires the mapping of continuous solutions [154] into the ON/OFF states of a Boolean network's

nodes. In order to achieve this, the time evolution of a function, determined by the rate functions and kinetic constants, has to be replaced with a discrete mapping of the node set into itself. Moreover, the rules of this self-mapping have to be governed by logical functions, connecting the binary states of interacting nodes. The dynamics of the resulting Boolean network is an ordered sequence of states of the network nodes, instead of the continuous time output of the ODE model. Having this in mind, let us find the conditions, which allow to perform the transition from a differential equations model to a Boolean system. In the following, we will first describe the passage of the continuous variables to discrete states and, in a second step, construct the logical functions representing the dynamics.

8.2.1 Stationary states of ODE system

The ODE model [154] described in section 5.3 uses arbitrary units for concentrations in all equations, since there are few data of actual protein concentrations. The kinetic constants represent the right timing of the processes. Solutions of the system show that the concentrations of the major proteins in general rise or decrease steeply.

To make the transition to a Boolean system, we first need to rescale the differential equations such that their solutions assume values between 0 (inactive) and 1 (maximum activity). This is a first step towards mapping these variables onto Boolean OFF/ON variables with values 0 and 1. The rescaling does not change the form of equations, it only affects the values of kinetic constants. To do this, let us divide all functions by their respective maximum value. For example, for $Slp1$ we introduce the new rescaled function $Slp1_1$=$Slp1Ampl$, where $Ampl = 2,1$ is the amplitude of the original solution. Rescaling all variables except M we obtain: The new values of parameters are shown in Table 8.1.

Next we map the continuous solution of the ODE model [154] into the discrete states of a Boolean network's nodes. Since in a Boolean model there is no continuous

$Cdc13_1$	$k_1 = 0.04$, $k'_2 = 0.03$, $k''_2 = 1$, $k'''_2 = 0.21$
$preMPF_1$	$k'_0 = 1.5$,$k''_0 = 1.17$, $k'''0 = 5$
$Ste9_1$	$k'_3 = 1$, $k''_3 = 21$, $J_3 = 0.01$, $k'_4 = 1.98$, $k_4 = 50.75$
$Slp1_{T1}$	$k'_5 = 0.002$, $k'_5 = 0.143$, $k'_6 = 0.048$, $J_5 = 0.20689$
$Slp1_1$	$k_7 = 0.429$, $k_8 = 0.119$, $J_7 = 0.0005$, $J_8 = 0.0005$
IEP	$k_9 = 0.16$, $J_9 = 0.01$, $k_{10} = 0.01$, $J_{10} = 0.011$ $k'_9 = 0.91$
$Rum1$	$k_{11} = 0.698$, $k_{12} = 0.01$, $k'_{12} = 0.99$, $k''_{12} = 4.35$
SK	$k_{13} = 0.1$, $k_{14} = 0.1$
M	$\mu = 0.005$
TF	$k_{15} = 3$, $k'_{16} = 1$, $k''_{16} = 2.9$, $J_{15} = 0.01$, $J_{16} = 0.01$
k_{wee}	$k'_{wee} = 0.115$, $k''_{wee} = 1$, $V_{iwee} = 1.45$, $V_{awee} = 0.25$, $J_{awee} = 0.01$, $J_{iwee} = 0.01$
k_{25}	$k'_{25} = 0.01$, $k''_{25} = 1$, $V_{i25} = 0.25$, $V_{awee} = 0.36$, $J_{a25} = 0.01$, $J_{iwee} = 0.01$, $J_{i25} = 0.01$
MPF	$k'''_{17} = 0.69$, $k_{17} = 1.5$, $k'_{17} = 1.3$, $k''_{17} = 1.5$, $k'''17 = 1.5$
Trimer	$k_{18} = 0.441$, $k'_{18} = 0.882$
σ	$k'_{19} = 1.5$, $k''_{19} = 0.147$, $K_{diss} = 0.001$

Tab. 8.1: Parameter values for the rescaled system of differential equations

time, but rather a sequence of switching events between two stationary states of the nodes, one needs to reduce (whereever possible) the initial system to a sequence of the evolution of stationary states. For this, one can use the results of a bifurcation analysis [154] of the transitions during the cell cycle. Thus, there are some variables ($Ste9$, $Slp1$, IEP) that are described by Goldbeter-Koshland (GK) functions [154] in the stationary state:

$$[Ste9_1] = G(k'_3 + k''_3[Slp1_1], k'_4[SK_1] + k_4[MPF_1], J_3, J_4) \tag{8.1}$$

$$[IEP_1] = 1/k'_9 G(k_9[MPF_1], k_{10}, J_9, J_{10}) \tag{8.2}$$

$$[Slp1_1] = [Slp1_{T1}]G(k_7[IEP_1], k_8, J_7/[Slp1_{T1}], J_8/[Slp1_{T1}]) \tag{8.3}$$

The characteristic properties of the GK function imply that its variable mainly resides in two limiting states: High and Low. The transition time between them is short (as the variables J are small). Therefore, we approximate them as Boolean (binary) variables.

It is easy to see that $Slp1_{T1}$ determines only the amplitude and the smoothness of the transition in (8.3), therefore, we neglect $Slp1_{T1}$ as a first step towards a Boolean

model and write

$$[Slp1_1] = G(k_7 IEP_1, k_8, J, J). \tag{8.4}$$

The states of the remaining variables can be described by Golbeter-Koshland functions, as well. For SK, while the equation for this variable is exponential, one can evaluate the right-hand part through a GK function with the stationary solution [154]:

$$[SK_1] = (k_{13}/k_{14})[TF] = (k_{13}/k_{14})G(k_{15}M_1, k'_{16} + k''_{16}[MPF_1], J_{15}, J_{16}). \tag{8.5}$$

Furthermore, there are three algebraic equations for TF (5.10), k_{wee} (5.11), and k_{25} (5.12) that contain Golbeter-Koshland functions. Here, again, the two limiting states of the GK function will be related to the binary ON/OFF version of the corresponding variables in the Boolean limit.

Finally, we will simplify the functional behavior for the $Cdc13_T$, $preMPF$, and $Rum1_T$, as well. Again we want to neglect the exact path of their transitions, keeping the limiting stationary states, eventually enabling us to take the limit of Boolean functions as a simplified description of the dynamics. Equations with the following requirements will allow us to take this limit:

a) In a small neighborhood of the switching point, the functional behavior can be approximated by an exponential rise

b) On the larger interval, it has a stationary solution with the steep transition between two limiting stationary states

c) This function converges to the Heaviside function in the limit of steep transition.

In the following we will see that the Michaelis-Menten dynamics fulfils these requirements, resulting in exact conformity of initial and final states and permitting a well controlled passage to a Boolean function. Let us start from the Michaelis-Menten

equation

$$\frac{dX}{dt} = k_1 \frac{1-X}{J_1+1-X} - k_2 \frac{X}{J_2+X}, \tag{8.6}$$

and first check the condition a). Expanding (8.6) in the neighborhood of switching points where $X << J_{1,2} << 1$ and keeping leading order terms yields

$$\frac{dX}{dt} = k_1 - \frac{k_2}{J_2} X. \tag{8.7}$$

This is a common equation of exponential growth/decrease. This allow us to take equations of exponential growth as an expansion of (8.7) in the neighborhood of switching ON/OFF points. For $Cdc13_T$, for example, the equation

$$\frac{d[Cdc13_{T1}]}{dt} = k_1 M - (k_2' + k_2''[Ste9_1] + k_2'''[Slp1_1])[Cdc13_{T1}] \tag{8.8}$$

is the expansion of the equation

$$\frac{d[Cdc13_{T1}]}{dt} = k_1 M \frac{1-[Cdc13_{T1}]}{J_1+1-[Cdc13_{T1}]} - (k_2' + k_2''[Ste9_1] + k_2'''[Slp1_1]) \times \\ \times [Cdc13_{T1}] \frac{[Cdc13_{T1}]}{J_2+[Cdc13_{T1}]}. \tag{8.9}$$

For illustration, in Fig. 8.1, this function is compared to the initial $Cdc13_{T1}$. Condition b) is satisfied as well, since the stationary states of (8.6) are described by a Goldbeter-Koshland function. Validity of condition c) is shown in the next section.

Thus, for the above equations we have a system of GK-functions which are responsible for the transitions between stationary states:

$$[Cdc13_{T1}] = G(k_1 M, k_2''[Ste9_1] + k_2'''[Slp1_1], J, J) \tag{8.10}$$

$$[preMPF_1] = G(k_0 k_{wee}[Cdc13_{T1}], [k_{wee_1}] + k_0''[k_{25_1}] + k_2' + \\ k_2''[Ste9_1] + k_2'''[Slp1_1]), J, J) \tag{8.11}$$

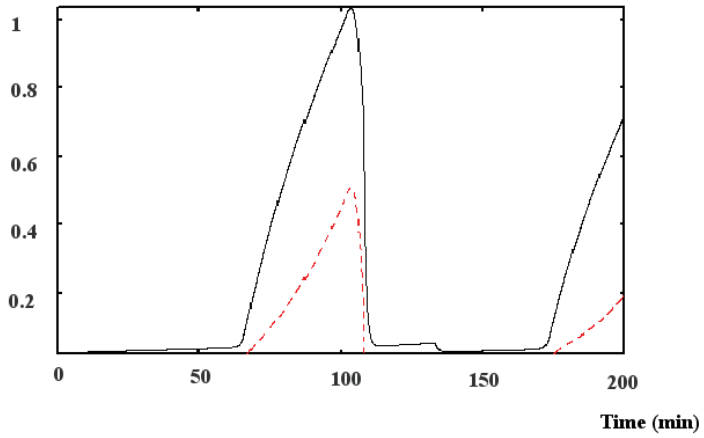

Fig. 8.1: Numerical simulation of (8.8) (black curve) and (8.10) (red curve). Note that the downward decrease occurs practically simultaneously.

$$[Rum1_{T1}] = G(k_{11}, k_{12} + k'_{12}[SK_1] + k''_{12}[MPF_1], J, J) \quad . \tag{8.12}$$

One can see in Fig. 8.1 that the new and initial functions start to grow and start to decrease at the same times, respectively. Note that the obtained substituted equations (8.10-8.12) play only a helper role and cannot be directly applied to the initial system of differential equations. The complete correspondence of the obtained system to the initial one is achieved by the limit transition shown in section 8.2.2.

Summarizing all information above we obtain the system of equations (5.10-5.12), (8.1-8.2), (8.4-8.5), (8.10-8.12), describing the stationary states of the corresponding variables. The next step is to perform a transition from the continuous functions to discrete functions. In functions with continuous time, the stepwise change corresponds to the Heaviside function.

8.2.2 Passage to Boolean variables

We now show that there is an exact passage from the function of Goldbeter-Koshland [72] to the indicator function (Heaviside step function). Let us remark that in (5.16) [154] the parameters a and b are functional variables, whereas c and d (in (5.10-5.12), (8.1-8.2), (8.4-8.5), (8.10-8.12) they are denoted as variables J) are usually fixed and small in all equations. The range of values of c, d varies from 0.001 to 0.01 and only one time for $Slp1$ it takes on the value 0.3. Very small parameters c and d mean that the enzyme-substrate complex is tightly bound and hardly dissociates. Thereby in [154] an assumption is made that the enzyme-substrate complexes involved are very stable [2]. For this reason, let us consider the behavior of the corresponding GK-functions in the limiting case $c \to 0$, $d \to 0$ while a and b take finite values.

First note that, both, numerator and denominator depend on d. Moreover, a is a numerator's factor. At the same time, c appears in a sum with the finite terms in the denominator, only (except at the point $b = a$). Therefore, we can assume, without loss of generality, that $c = 0$ and consider below

$$G(a, b, 0, d) = \frac{2ad}{b - a + ad + \sqrt{(b - a + ad)^2 - 4ad(b - a)}}.$$

Using the Taylor expansion of the square root in the denominator and neglecting higher powers of d, we obtain for all points $a \neq b$

$$G(a, b, 0, d) = \frac{2ad}{b - a + ad + |b - a| - ad\frac{b-a}{|b-a|}}. \tag{8.13}$$

There are two possible cases:

- a) if $b - a > 0$ then $|b - a| = b - a$ and (8.13) takes a form

$$G(a, b, 0, d) = \frac{2ad}{b - a};$$

- b)if $b-a<0$ then $|b-a|=-(b-a)$ and (8.13) is simply G(a,b,0,d)=1.

This implies that there is a passage to the limit from Goldbeter-Koshland function $G(a,b,c,d)$ to the Heaviside function $\theta(a-b)$:

$$\lim_{c,d\to 0} = \theta(a-b) = \begin{cases} 0, & a<b, \\ 1, & a>b. \end{cases}$$

Thus, in this limit, the variable a plays a role of an activator input and the variable b is an inhibitor input. The output is active/inactive (its Boolean value is equal to one/zero) if the total value of activator inputs is larger/smaller than the total value of inhibitor inputs. Thus, the Goldbeter-Koshland-function converges to the Heaviside function in the limit of steep transitions.

8.2.3 Logical Boolean functions

Let us now rewrite the system of equations in the limit $Js\to 0$ (J_3, J_5, J_7, J_8, J_10, J_{16}, J_{awee}, J_{iwee}, J_{i25}, J_{a25} and Js in (20), (26-28) equations) as

$$[Ste9_1]=\theta(k_3'+k_3''[Slp1_1]-k_4'[SK_1]-k_4[MPF_1]) \tag{8.14}$$

$$[IEP_1]=\theta(k_9[MPF_1]-k_{10}) \tag{8.15}$$

$$[SK_1]=(k_{13}/k_{14})\theta(k_{15}M_1-k_{16}'-k_{16}''[MPF_1]) \tag{8.16}$$

$$[TF_1]=\theta(k_{15}M-k_{16}'-k_{16}''[MPF_1]) \tag{8.17}$$

$$[k_{wee_1}]=k_{wee}'+(k_{wee}''-k_{wee}')\theta(V_{awee}-V_{iwee}[MPF_1] \tag{8.18}$$

$$[k_{25_1}]=k_{25}'+(k_{25}''-k_{25}')\theta(V_{a25}[MPF_1]-V_{i25}) \tag{8.19}$$

$$[Cdc13T_1]=\theta(k_1M-k_2''[Ste9_1]-k_2'''[Slp1_1]) \tag{8.20}$$

$$[preMPF_1]=\theta(k_{wee}[Cdc13_{T1}]-[k_{wee_1}]-[k_{25_1}]-k_2'-k_2''[Ste9_1]- \tag{8.21}$$
$$-k_2'''[Slp1_1])$$

$$[Rum1_{T1}]=\theta(k_{11}-k_{12}-k_{12}'[SK_1]-k_{12}''[MPF_1] \tag{8.22}$$

$$[Slp1_1] = \theta(k_7[IEP_1] - k_8). \tag{8.23}$$

Let us add two simplifications to the equations (8.16) and (8.18-8.19). In equation (8.16), the coefficient $k_{13}/k_{14} = 1$ and thus can be neglected. In equation (8.18), k_{wee_1} can have two possible values: 0.115 and 1. The first one can be reduced to 0 since it does not change the behavior of the system. It is analogous for k_{25}

$$[k_{wee_1}] = \theta(V_{awee}, V_{iwee}[MPF_1], J_{awee}, J_{iwee}) \tag{8.24}$$

$$[k_{25_1}] = \theta([MPF_1] - V_{i25}) \tag{8.25}$$

$$[SK_1] = \theta(k_{15}M - k'_{16} - k''_{16}[MPF_1]). \tag{8.26}$$

Thus, we have a system of ten equations, where all variables, except MPF and M can take values 0 or 1. MPF and M cannot be described in this formalism. MPF is represented by an algebraic equation which cannot be reduced to the GK-function. Taking a closer look, the solution of MPF does not reach a simple stationary state, instead there are three typical states of MPF in the system ($preMPF$, $Rum1$, $Cdc13$) – OFF, intermediate and high activation.

1) If $Cdc13 = 0$ then $MPF = 0$, independently of the states of $preMPF$ and $Rum1$.

2) If $Cdc13 = 1$ and $preMPF = 1$ then $MPF = 0.14$, independently of the state of $Rum1$. This corresponds to an intermediate level, when $preMPF$ prevents high excitation.

3) If $Cdc13 = 1$, $preMPF = 0$, and $Rum1 = 1$, then $MPF = 1$, with its value slightly decreasing to $MPF = 0.93$ if $Rum1 = 0$. This corresponds to a high level of activation, when MPF is activated by $Cdc13$ and this activation is not reduced by $preMPF$.

Let us reformulate these rules in the following. Assume there are two variables – MPF and $MPF2$. The first one is activated by $Cdc13$. For activation of the

second variable $MPF2$, one assumes that MPF has to be present as a low-level and $preMPF$ should be inactive. Thereby, one needs to rewrite the system of equations (8.14), (8.20-8.22) taking into account which level of excitation of MPF is crucial for each particular variable.

$$[MPF_1] = \theta(Cdc13_{T1}) \tag{8.27}$$

$$[MPF2] = \theta(MPF_1 - [preMPF_1]) \tag{8.28}$$

$$[IEP_1] = \theta([k_9[MPF_1] - k_{10}) \tag{8.29}$$

$$[TF_1] = \theta(k_{15}M - k'_{16} - k''_{16}[MPF2_1]) \tag{8.30}$$

$$[Rum1_{T1}] = \theta(k_{11} - k_{12} - k'_{12}[SK_1] - k''_{12}[MPF_1]) \tag{8.31}$$

$$[k_{wee_1}] = \theta(V_{awee} - V_{iwee}[MPF_1]) \tag{8.32}$$

$$[k_{25_1}] = \theta([MPF_1] - V_{i25}) \tag{8.33}$$

$$[SK_1] = \theta([TF]) \tag{8.34}$$

$$[Ste9_1] = \theta(k'_3 + k''_3[Slp1_1] - k'_4[SK_1] - k_4([MPF_1])) \quad . \tag{8.35}$$

Second, as in the model based on differential equations the cell mass M takes a special role in the present model. The solution [154] treats it during a cell growth as an independent variable, which is described by an exponential growth function (5.9). Thus, the variable M corresponds to a time in this system, which drives the evolution between stationary states [154]. In the system, M directly influences $Cdc13$ and TF. As soon as M reaches a threshold value, it activates $Cdc13$ and induces the sequence of consecutive transitions between stationary states. For TF it plays a role of constantly positive input, TF is always active unless MPF has a high activity.

As a criterium for the end of the cycle, Novak et al. [154] determine when the cell divides by monitoring the values of the other variables. When these chosen variables have certain values that indicate the end of the cell cycle, the current value of M is

divided by two manually, as at the end of mitosis the cell divides into two daughter cells of approximately equal masses. Subsequently, M continues its exponential growth, again.

Following this strategy, one needs to distinguish M between two principal different values – M and $2M$ in the Boolean model. Here M works at the beginning of the cell cycle as a trigger of switching events, whereas $2M$ play a role of an indicator for the end of the cell cycle. Correspondingly, M becomes $2M$ at the end of mitosis, when $Slp1$, $Ste9$ and IEP all have high concentrations.

Thus, one can add the following Boolean rule:

$$M = \theta(2M - [Ste9_1][Slp1_1][IEP_1]) \tag{8.36}$$

$$2M = \theta(M[Ste9_1][Slp1_1][IEP_1] - 2M) \tag{8.37}$$

Thus, we have a system of equations (8.27-8.37), where each variable can take values 0 or 1, only. It is easy to simplify this system, reducing the kinetic coefficients to 0 or 1 and adding thresholds. Consider, for example, $Cdc13_T$. In Table 8. 2, based on equation (8.20), we show all possible cases for $Cdc13$. In a more compact form, where the kinetic constants are reduced to 1, these rules become

$$[Cdc13T_1] = \theta(M - [Ste9_1] - [Slp1_1]). \tag{8.38}$$

Repeating the same procedure for all variables, we obtain the system of equations:

$$[preMPF_1] = \theta(k_{wee} + [Cdc13_{T1}] - 1 - [k_{25_1}] - [Ste9_1] - [Slp1_1])) \tag{8.39}$$

$$[Slp1_1] = \theta([IEP_1]) \tag{8.40}$$

$$[TF_1] = \theta([M] + [2M] - [MPF2_1] \tag{8.41}$$

$$[IEP_1] = \theta([[MPF_2]) \tag{8.42}$$

Number	M	$Ste9$	$Slp1$	$Cdc13$
1	0	0	0	0
2	1	0	0	1
3	1	1	0	0
4	1	1	1	0
5	0	1	0	0
6	0	1	1	0
7	1	0	1	0
8	1	1	1	0

Tab. 8.2: Boolean rules for variable $Cdc13$.

$$[Rum1_{T1}] = \theta(0.5 - [SK_1] - [MPF_1]) \tag{8.43}$$

$$[k_{wee_1}] = \theta(0.5 - [MPF_1]) \tag{8.44}$$

$$[k_{25_1}] = \theta([MPF_1] - 0.5) \tag{8.45}$$

$$[SK_1] = \theta([TF_1]) \tag{8.46}$$

$$[Ste9_1] = \theta([Slp1_1] - [SK_1] - [MPF_1]) \tag{8.47}$$

$$M = \theta(2M + 3 - [Ste9_1] - [Slp1_1] - [IEP_1]) \tag{8.48}$$

$$2M = \theta(M + [Ste9_1] + [Slp1_1] + [IEP_1] - 3 - 2M) \tag{8.49}$$

8.3 Boolean model

We now have a system of algebraic equations (8.38-8.47), which describe the switch-like transitions between stationary states, plus Boolean equations (8.27-8.28),(8.48-8.49) for M and MPF. Note that in this discrete system, no information about continuous time is present any more, except the sequence of events. To obtain this discrete dynamical sequence, we iteratively solve the system. We start from the known initial conditions [154]: $2M = 1$, $Slp1_1 = 1$, IEP_1=1, $Ste9_1 = 1$, $k_{wee1} = 1$, with all other variables being 0. Following the terminology of Boolean models, each variable is represented by a node. The network of nodes is shown in Fig. 8.2. Each node i has only two states, $S_i(n) = 1$ (active) and $S_i(n) = 0$ (inactive). The index n is the

number of iterations. The iterative solution of the system has the following general form:

$$S_i(n+1) = \theta\left[\text{sign}\left(\sum_k T_{ik}S_k(t) + Q_i\right)\right], \tag{8.50}$$

with the sign function

$$\text{sign(x)} = \begin{cases} 1, & x > 0 \\ 0, & x = 0 \\ -1, & x < 0 \end{cases} \tag{8.51}$$

and the Heaviside function

$$\theta(x) = \begin{cases} 1, & x > 0 \\ 0, & x \leq 0. \end{cases} \tag{8.52}$$

All nodes are updated synchronously, which corresponds to the iteration of the full dynamical system. The interaction matrix T_{ik} and the state vector Q_i determine the transition rule between states.

The specific values of the interactions T_{ik} are determined as follows. All interactions between a pair of nodes are defined by an interaction strength given as the elements T_{ik} and an activation threshold Q_i. Positive (negative) arguments of the θ-functions have $T_{ik} = 1$ ($T_{ik} = -1$). $T_{ii} = 1$ for M, this rule is true only for M node, since it is described by a growing exponential function.

The resulting matrix T_{ik} and vector Q_i have the following form:

$$T_{ik} = \begin{pmatrix} & 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10 & 11 & 12 & 13 & 14 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 \\ 2 & 1 & 0 & 0 & 0 & -1 & 1 & 0 & -1 & -1 & 0 & 0 & 0 & 0 & 0 \\ 3 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 4 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 5 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 6 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 7 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 1 & -1 & 0 \\ 8 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 9 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & -1 & 0 & 0 & 0 \\ 10 & 0 & 0 & 0 & -1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 11 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 12 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 1 & 1 & 0 \\ 13 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 14 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \end{pmatrix}$$

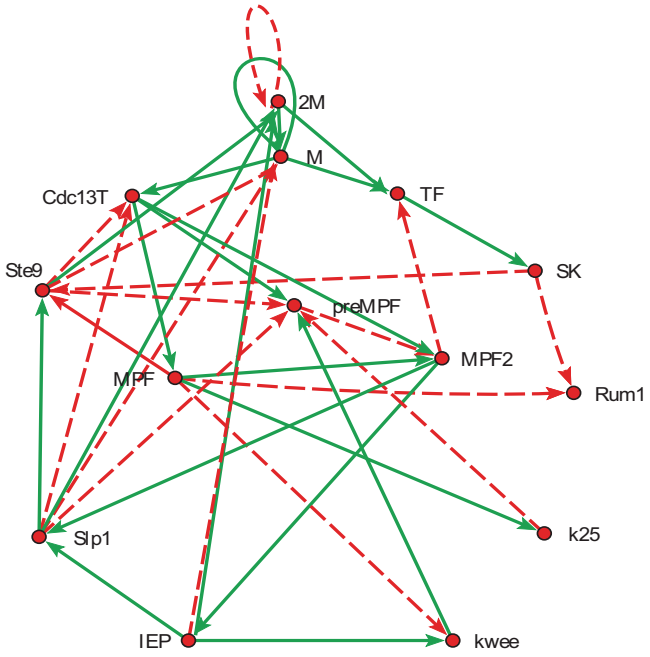

Fig. 8.2: Interaction network of the Boolean model. Green links correspond to T_{ik}=+1 and red links to ones to T_{ik}=-1

$$Q_i^T = \begin{pmatrix} 0 & -1 & 0 & 0 & -0.5 & 0.5 & 3 & 0 & 0 & 0 & 0 & -3 & 0 & 0.5 \end{pmatrix}$$

where numbers in the first raw and column denote: 1: Cdc13T, 2: preMPF, 3: MPF, 4: MPF2, 5: k25, 6: k_{wee}, 7: M 8: Slp1, 9: Ste9 10: TF, 11: SK, 12: 2M, 13: IEP, 14: Rum1.

8.4 Results of Boolean simulation of the fission yeast cell cycle

First we run the model described in the previous section with the initial conditions [154]. The temporal evolution of the protein states is presented in Table 8.3. One can see that the iterative solution of the system (8.27-8.28) (8.38-8.49) is the switching between unstable stationary states, which coincide with the corresponding evolution in the ODE model. The final state is a stable stationary state of the system. One

Number of iteration	$Cdc13_T$	$preMPF$	$MPF1$	$MPF2$	k_{25}	k_{wee}	M	$Slp1$	$Ste9$	TF	SK	$2M$	IEP	Rum1
1	0	0	0	0	0	1	0	1	1	0	0	1	1	1
2	0	0	0	0	0	1	1	1	1	1	0	0	0	1
3	0	0	0	0	0	1	1	0	1	0	1	1	0	1
4	0	0	0	0	0	1	1	0	0	1	1	0	0	0
5	1	0	0	0	0	1	1	0	0	1	1	0	0	0
6	1	1	1	0	0	1	1	0	0	1	1	0	0	0
7	1	1	1	0	1	0	1	0	0	1	1	0	0	0
8	1	0	1	0	1	0	1	0	0	1	1	0	0	0
9	1	0	1	1	1	0	1	0	0	1	0	0	0	0
10	1	0	1	1	1	0	1	0	0	0	0	0	1	0
11	1	0	1	1	1	0	1	1	0	0	0	0	1	0
12	0	0	1	1	1	0	1	1	0	0	0	0	1	0
13	0	0	0	1	1	0	1	1	0	0	0	0	1	0
14	0	0	0	0	0	1	1	1	1	0	0	0	1	1

Tab. 8.3: Temporal evolution of protein states for the cell-cycle control network.

notices that the initial and end states are identical except for the activation of the nodes M and $2M$. The update of nodes M and $2M$, keeping all other nodes in the same states, starts the new cycle. This cycling of the model is similar to the realization of cycling in the original model of differential equations.

Let us briefly summarize our coarse-graining strategy that we followed in this chapter. In Fig.8.3, we show consecutive abstractions of the model for the $Ste9$ and IEP variables as an example. For this we first plot the dynamics of the differential equations model, then the evolution between the stationary states solutions of the ODE model, and finally the sequence of states obtained from the iterated Boolean network model.

In the next step we run the model starting from each of the 2^{15} possible initial states. We find that from all initial states 67% flows into one big attractor. This attractor is the same stable stationary state that one obtains starting with biological conditions described above.

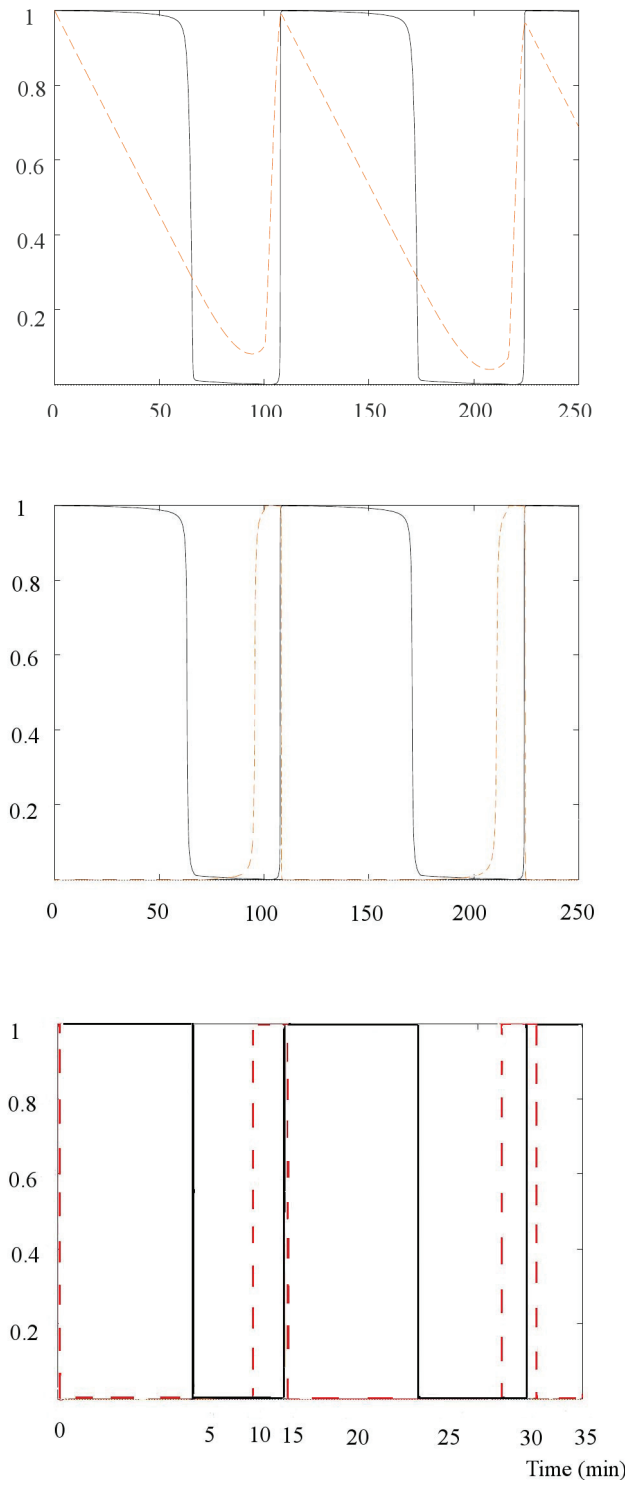

Fig. 8.3: Upper plot: numerical simulation of system of differential equations for IEP (red curve) and $Ste9$ (black curve). Middle plot: numerical simulation of stationary states for IEP (red curve) and $Ste9$ (black curve). Bottom plot: Boolean solution in steps for IEP (red curve) and $Ste9$ (black curve)

8.4.1 Mutations

Let us also compare the behavior of mutants. We model two mutations – Wee^- and $Wee^-Cdc25\triangle$ described in [154]. In Boolean models one cannot distinguish between reduced activity and no activity. This is why we model Wee^- as $Wee\triangle$ in both cases. We run the model starting with wild type initial conditions, but with deleted nodes k_{wee}, and in the second case k_{wee} and k_{25}. In both mutations, the number of steps is reduced to 12, as compared to 14 in the wild type cell cycle described in the previous section. This suggests that the cell can divide at a smaller size than the wild type, where both mutations are viable. Our results are in accordance with the predictions of the earlier differential equation model [154].

8.4.2 Comparison with an existing Boolean model for the fission yeast cell cycle

It is interesting to compare this model with our original Boolean model, introduced in chapter 6, for the fission yeast cell cycle [43] that was built on known biochemical interactions between proteins, only. Both models are quite similar and have the same connections between homologue nodes. Their dynamics matches the wild type signaling sequence during the cell cycle. The difference is that in the current model the nodes $Cdc13$, $preMPF$, $MPF1$, $MPF2$ correspond to only two nodes Cdc2/Cdc13 and Cdc2/Cdc13* in [43]. So in the model [43], the complex Cdc2/Cdc13 can have two levels of activation - medium and high. The intermediate level corresponds to sole activation of the Cdc2/Cdc13 node, whereas a high level of activation is represented by activation of both, Cdc2/Cdc13 and Cdc2/Cdc13* .The last one plays the role of a dephosphorylated Cdc2/Cdc13, that closely corresponds to MPF in [154]. In the current model, the node MPF was separated into two nodes $MPF1$ and $MPF2$ as well, to distinguish different levels of MPF activation.

The formulas responsible for evolution of proteins are similar in a sense that in

both a threshold Boolean function of update is used. Although there are also some differences. Therefore, in our original Boolean network model of the fission yeast cell cycle [43], proteins remain active if the corresponding node was not switched-off by other incoming inhibiting signals. This rule means, that if the protein was activated it requires some other signals to change its state. Whereas in the current model one needs to have always a positive incoming signal in order to keep the protein in its active form.

8.5 Discussion and Conclusion

In this chapter, our aim is to make a connection between two successfully used methods - the ODE and the Boolean models for predicting properties of a real biological process. In particular, we show a possible limit transition between the ODE and the Boolean model for the fission yeast cell cycle. For this purpose the known ODE model [154] of fission yeast has been chosen.

In order to do the transition, we start with scaling: The differential equations are rewritten in a such way that the solutions of equations reach 1 in their maximum values. Then the obtained equations are transformed in a limiting procedure to Boolean functions. Firstly, Michaelis-Menten equations and equations with switch-like behavior can be directly reduced to Boolean functions. Secondly, a set of equations with sigmoidal transfer functions can be replaced with Michaelis-Menten equations without changing the sequence of states through which the system evolves. Thirdly, there are also some cases that cannot be reduced to the two previous ones. It happens when the variable is described by a constantly growing function or a function which has distinctly different levels. In this case we propose in the Boolean model to substitute those variables with two labeling intermediate and high activity of it. Finally, all continuous solutions of equations are mapped into ON/OFF states of Boolean network

and the transition between states are described by Boolean functions [44].

This Boolean model reproduces the results of the initial NT ODE model [154]. In particular, starting with initial conditions as in [154], the system evolves through the same sequence of states. The second evidence of similar behavior of the ODE and the Boolean model is the robustness to the initial conditions. The Boolean model has a dominant attractor (67%), attracting most of the trajectories, starting from different initial conditions. The dominant attractor coincides with initial biological conditions of the system. The ability to model mutations in the Boolean approach additionally confirms a good correspondence between the ODE and the Boolean model.

We find that the transition to a Boolean model is possible for differential equations, which have monotonic sigmoidal functions with distinct upper and lower asymptotes. In particular, firstly, in our case Michaelis-Menten equations are reduced to S-shaped GK-functions which have the necessary asymptotes [69, 71]. This function works as a switch between the cases when parameters are defined as the upper or lower asymptote and the target control function corresponds to the maximal or basal rate of biochemical processes. Secondly, here substituting some equations that have monotonic sigmoidal functions on the right-hand-side with Michaelis-Menten functions, we also find, that the exact form of the sigmoidal function does not strongly influence the behavior of the system. The comparison of the current model with a previous Boolean model for fission yeast reveals that they both have a similar set of variables (proteins) and similar Boolean functions responsible for update.

Our results also confirm the idea that some molecular control networks are so robustly designed that timing is not a critical factor [25]. In our case it is possible to reproduce the main results of [154] without including time, but repeating the right sequence of events. It supports the idea that the Boolean approach could contain sufficient information. Thereby one needs less information about the system, the knowledge about reactions on the level of activation/inhibition is sufficient, which

eliminates the problem of finding the right kinetic constants. Another advantage is the low computational cost of Boolean networks. The problems one meets working with the Boolean approach are that it is sometimes difficult to reduce the concentration level of some proteins only to ON/OFF states. Sometimes there are intermediate states of concentration which need to be separated from high concentration. In this case two methods are possible, described in the discussion part of chapter 7. One is, as we implemented it here, to divide this variable into two and to perform as two different nodes in a system. Doing this, one needs to take into account the differences in influences of this protein when it has intermediate and high concentration. Another solution for a such situation could be the introduction of two discrete levels of concentration that the protein can have, for example 1 for intermediate and 2 for high concentration, as it has been already mentioned in chapter 7.

We would like to note that the ODE and the Boolean approach are both useful methods. The advantage of the ODE approach is that it provides detailed information about the system at any given time in contrast to the Boolean method, which reproduces only the right sequence of events. However, the costs for this information are the following. One needs to have exhaustive information about the reactions, where the most difficult part is to find the right kinetic constants. Also it demands more computational costs to find the solution of the system. One could say that the ODE approach is appropriate when the system is well studied and it is necessary to make a detailed study of all reactions that take place. On the other hand if the task is to understand the main principles of some process and one has less information, the Boolean approach is very suitable to use.

In the next, last chapter 9 we recapitulate the main results of this thesis with a prospectus on further developments.

9. SUMMARY AND OUTLOOK

Gene and protein regulatory networks guide all functions in cells and are very complex. Predicting the dynamics of these networks is a central task of systems biology. Although nowadays cell-wide, or organism-wide, models of genetic and molecular interactions appear out of reach, predictive models of single pathways and small modular molecular networks of living cells have been studied with great success and are a matter of active research.

For predicting the dynamics of these networks various experimental and mathematical techniques have been developed. A common challenge for the most widespread mathematical approaches is the demand of parameters governing the behavior of such networks over time. For instance, using the most widespread method, differential equations, very detailed information on concentrations and kinetic constants is necessary.

In this thesis, we verified that a minimalistic Boolean approach allows to build satisfactory predictive models that reproduce the sequence of protein activations with no demand on kinetic constants. We demonstrated this on an example of a general model of apoptosis for human cells and a model for the fission yeast cell cycle (*Schizosaccharomyces Pombe*).

First, we have constructed a Boolean model for apoptosis in human cells consisting of 70 proteins. The dynamical properties of this model indicate that apoptosis is a robust irreversible process, i.e. if one of the apoptosis pathways has been triggered,

in spite of anti-apoptotic proteins, in most cases the cell dies. The influence on apoptosis rate has been tested on the 8 most important proteins. The observed changes qualitatively reproduce the experimental data [40, 90, 91, 224], thereby providing a verification of the model.

Further, we have constructed the Boolean network model for the fission yeast cell cycle and found a number of interesting results. We showed that the model, constructed solely on the basis of the known biochemical interaction topology, reproduces the known activity sequence of regulatory proteins along the cell cycle of the living cell. The dynamical properties of the model indicate that the biological dynamical sequence is robustly implemented in the regulatory network, with the biological stationary state G1 corresponding to the dominant attractor in state space, and with the biological regulatory sequence being a strongly attractive trajectory. Therefore, it is unlikely that the process will deviate from the initial one.

The validity of the model was tested on a large number of mutations. The different types of mutations were modeled as the following. The loss-of-function mutations were implemented by deleting the corresponding node (nodes in case of double and triple mutants correspondingly). All loss-of-function mutations were reproduced in the frames of the model except a small number. These are not reconstituted because of the dramatic simplifications that were made on the interactions between some proteins. For overexpressed mutations the additional constant positive input to activation rule was added. The reproduction of some overexpressed mutants covers not all known mutants because of the limitation of different activation states to ON/OFF states in a Boolean model.

Summarizing, the constructed Boolean network model reproduces 30 mutants, which is 70% of known mutations in frames of involved proteins (the existing ODE models were tested for up to 22 mutations). Thus, the model covers the main mechanisms of the process successfully and allows to make predictions on the modifications

such as mutants that underlie changes in the phenotype.

The comparison of the Boolean network model of the fission yeast cell cycle with its randomized version showed that these have remarkably different dynamical properties. This result suggests that the research of random Boolean networks has a limited potential, since it provides a very mean picture of the process, averaging all possible networks, whereas real biological regulatory networks can have very specific topology which deviates from a mean significantly. Therefore, the dynamical properties of the system will be also different than in a corresponding RBN model.

The overall results obtained from our Boolean network model are in accordance with the existing ODE models of fission yeast. It is essential to remark that nowadays there is no general ODE model for the fission yeast cell cycle that would cover all details of the process simultaneously and each of the ODE models is focused on this or that aspect of the process. The main advantage of our Boolean model is that we were able to drop 47 kinetic constants that were necessary in the ODE approach and, while doing so, still reproduce the biological sequence of protein activation. The Boolean network model allows to include different aspects of fission yeast cell cycle in one model and reproduces the maximal number of mutations. The limitations of the Boolean model are that it is not able to reproduce the exact timing and temperature-sensitive mutants due to its discrete nature.

In the last part of the thesis, we showed a possible limit transition between the most widespread method – ODE and Boolean models on an example of the fission yeast cell cycle. We mathematically derived a Boolean model from an existing ODE model for the fission yeast cell cycle and tested it on reproduction of the results and dynamical properties of the system. Our investigations suggest that the obtained Boolean model reproduces the results of the initial ODE model: It evolves through the same sequence of states, robustly to the initial conditions and reproduces the

same mutations. Thereby, we found that the transition to a Boolean model is possible for differential equations, which have monotonic sigmoidal functions with distinct upper and lower asymptotes. In particular, firstly, in our case Michaelis-Menten equations are reduced to S-shaped Goldbeter-Koshland functions which have the necessary asymptotes [71]. This function works as a switch between the cases when parameters are defined as the upper or lower asymptote and the target control function corresponds to the maximal or basal rate of biochemical processes. Secondly, substituting some equations that have monotonic sigmoidal functions on the right-hand-side with Michaelis-Menten functions, we also found that the exact form of the sigmoidal function does not strongly influence the behavior of the system. The comparison of the current model with our biologically constructed Boolean model for fission yeast reveals that both have a similar set of variables (proteins) and similar Boolean functions responsible for updates.

Thus, we prove that the Boolean network model can be successfully used for modeling biological processes on example of the fission yeast cell cycle and apoptosis. We showed that Boolean networks easily cover different aspects of biological process in one model with no need of building a series of models fitting kinetic constants and concentrations as in ODE models. A problem while working with the Boolean approach is that it is not always possible to reduce the concentration level of some proteins to ON/OFF states only. The possible development of Boolean models includes introduction of different discrete levels of states, distinguishing not only between 0 or 1, but 0, 1, 2 etc, as it was proposed by [198]. Another option is to represent one node with different levels of activation with two or more nodes. This second approach has been implemented in the current model. In cases when it is not possible to discretize the state of some variable, one can use a hybrid method, which combines ODE and the Boolean network approach. In a hybrid method some components, which have

switch-like behavior and thereby are easy to discretize, are modeled as Boolean networks. Other components are modeled with differential equations. This combining method simplifies the system, reducing the number of necessary kinetic constants and therefore allows to include a larger number of components and model different types of components (discretizable and non-discretizable). The ability to model the fission yeast cell cycle with the Boolean network method can be also interpreted in this way that the nature of this process is to some certain degree discrete. It also supports recent research results [25] that some molecular networks are so robustly designed that timing is not a critical factor, and one can drop the requirement of accurate reproduction of time and reconstruct just a sequence of states.

Qualitative discrete modeling has been successfully applied to gene/protein regulatory networks for building predictive models in particular, modeling mutants [7, 55, 56, 82]. However, the constructed Boolean network model for the fission yeast cell cycle is the first one that reproduces overexpressed mutations and such a large number of double and triple mutations. The astonishing fact is that in order to grasp the comprehensive dynamical behavior we use very simple threshold rules of activation compared to complex logical rules that were implemented for other Boolean models. Such a network is a starting point for further spatio-temporal dynamical models. Summarizing, Boolean network models can be used as a rather good first approximation for modeling biological processes. This approach is able to catch the basic dynamical properties of a process and to understand the main mechanisms. Further, Boolean models do not demand kinetic constants that cause problems in an ODE approach. As soon as one comprehends the main principles of the process, it is much easier to build more complicated and detailed models, such as ODE, in cases when more precise analysis is needed.

Therefore, we encourage further modeling experiments with a Boolean approach.

This may prove a quick approach for predicting biologically relevant dynamical features of genetic and protein networks in living cells. A qualitative date may be sufficient to comprehend the controlling mechanism of the system.

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9.1 Publications

M.I. Davidich, S. Bornholdt (2009) Boolean model for fission yeast cell cycle describing mutations. Article is under preparation.

M.I. Davidich, S.Bornholdt (2008) The transition from differential equations to Boolean networks: A case study in simplifying a regulatory network model. Journal of Theoretical Biology 255 (3): 269-277.

M.I. Davidich, S.Bornholdt (2008) A Boolean model predicts cell cycle sequence of yeast PLOS ONE, 2008, 27; 3(2):e1672.

9.1.1 Conferences, presentations

H. Fellman, M. I. Davidich "Coarse-graining modular Boolean networks" paper in proceedings, Santa Fe Complex systems summer School 2008.

M.I. Davidich, S.Bornholdt "Robustness of the fission yeast cell cycle network", ICSB2007, Long Beach, USA, 2007. Poster presentation and paper in proceedings, F04.

M.I. Davidich, S. Bornholdt Talk "How general is the Boolean approach for predictive models? A case study of the yeast cell cycle." NBIC-ISNB, Amsterdam, Netherlands, 2007. Abstract in proceedings, p.33.

M.I. Davidich "Boolean networks: the main concepts and use for real biological systems". Invited lecture, scientific seminar, Humbolt University, Berlin, Germany, 2007.

M.I Davidich "How general is the Boolean network approach for predictive models?". Invited lecture, scientific seminar, University of Nijmegen, Nijmegen, Netherlands, 2007.

M.I. Davidich, S. Bornholdt Talk "How general is the Boolean approach for predictive models? A case study of the yeast cell cycle " DPG-Tagungen", Regensburg,

Germany, 2007. Abstract in proceedings, p.81.