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Bifurcation analysis of the regulatory modules of the mammalian G_1/S transition

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ABSTRACT

Motivation: Mathematical models of the cell cycle can contribute to an understanding of its basic mechanisms. Modern simulation tools make the analysis of key components and their interactions very effective. This paper focuses on the role of small modules and feedbacks in the gene–protein network governing the G_1/S transition in mammalian cells. Mutations in this network may lead to uncontrolled cell proliferation. Bifurcation analysis helps to identify the key components of this extremely complex interaction network.

Results: We identify various positive and negative feedback loops in the network controlling the G_1/S transition. It is shown that the positive feedback regulation of E2F1 and a double activator–inhibitor module can lead to bistability. Extensions of the core module preserve the essential features such as bistability. The complete model exhibits a transcritical bifurcation in addition to bistability. We relate these bifurcations to the cell cycle checkpoint and the G_1/S phase transition point. Thus, core modules can explain major features of the complex G_1/S network and have a robust decision taking function.

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INTRODUCTION

Modeling of the eukaryotic cell cycle is particularly relevant due to the role of cell cycle dynamics in tumor development. However, neither is the mammalian cell cycle well understood nor do precise mathematical models of the whole cycle exist. Nevertheless, a lot of work has already been done, but the focus of the research concentrates mainly on small functional subsystems (Bai *et al*., 2003; Kel *et al*., 2000; Obeyesekere *et al*., 1997).

In contrast, quite detailed and powerful models of the yeast cell cycle exist (Chen *et al*., 2000; Tyson *et al*., 2001). The authors translate acquired biological knowledge into systems of differential equations and characterize signatures of the cell cycle such as checkpoints using bifurcation theory. These models are able to predict dynamics of mutants and provide testable hypotheses.

THE G_1/S MODEL

In this paper, we study two key points during the G_1/S transition of the mammalian cell cycle: the restriction point, R, and the phase transition point between the two cell cycle phases. For this purpose, we utilize bifurcation analysis and discuss points of interest in terms of transcritical and saddle node bifurcations. In particular, we show that the complex network can be dissected into small elementary modules.

The eukaryotic cell cycle is usually divided into four phases ($\rightarrow$ G_1 $\rightarrow$ S $\rightarrow$ G_2 $\rightarrow$ M $\rightarrow$): the S-phase (synthesis—DNA replication), the M-phase (mitosis, chromosome separation) and two gaps between them, G_1 and G_2. Two points during the loop are of particular importance, so-called checkpoints, one before the G_1/S transition and the other transition before the G_2/M transition. These checkpoints block the entry to the next stage if the previous step has not been completed or if the signal is insufficient for progress.

We present a model of the G_1/S transition in mammalian cells, which includes a set of proteins and their regulatory gene network. It is based on a model proposed by Kel *et al*. (2000) and includes the core proteins responsible for progression from the G_1 phase to the S-phase of the cell cycle:

- pRB (retinoblastoma), tumor suppressor from the family of pocket proteins (pRB, p107, p130).
- E2F1, transcription factor targeting genes that regulate cell cycle progression (cyclins and cyclin-dependent kinases, E2F1, histones, Myc and Myb-transcription factors, DNA replication proteins).
- AP-1, family of transcription factors (dimers of Fos and Jun proteins) that mediate mitogenic signals.
- Cyclin D/cdk4,6, cyclin E/cdk2, complexes, of which the levels vary during the cell cycle, characterizing the G_1- and S-phases.

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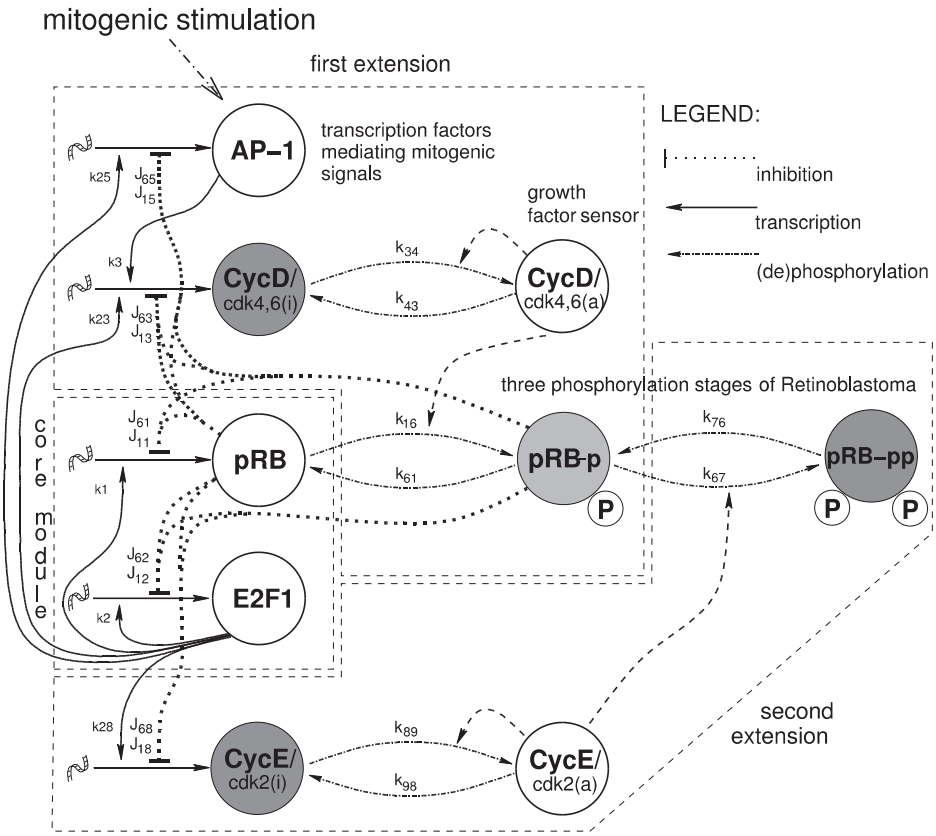

Fig. 1. Transition from the G_0-phase to the S-phase under the influence of growth factors. The phase-dependent phosphorylation of pRB is carried out successively by the complexes of cyclins D, E and cyclin-dependent kinases cdk4, 6 resp. cdk2.

The aim of this model is to verify previous results in an extended model, to draw conclusions about the role of feedback loops and to characterize the restriction point, R, and the transition point at G_1/S in terms of bifurcation analysis. More precisely, the restriction point, R, will be characterized as a transcritical bifurcation, and the G_1/S transition by a saddle node bifurcation and bistability ('toggle-switch'). The critical point of the transcritical bifurcation is often interpreted as a threshold value, consistent with the intuitive understanding of a restriction point. We can quantify the duration and strength of the mitogenic stimulation that are needed to cause the launching of the cell cycle.

COMPARISON WITH THE YEAST CELL CYCLE MODEL

As mentioned above, sophisticated yeast cell cycle models exist (Chen *et al*., 2000; Tyson *et al*., 2001). We compare briefly the basic cellular mechanisms and justify the need for a new model for higher eukaryotes. In particular, we discuss the cell-size dependence of proliferation.

Saccharomyces pombe In yeast, only one cyclin-dependent kinase, cdk1, also called cdc2, governs the cell cycle in complexes with different cyclin units. The models are based on basically one core dimer, cdk1/cdc13 (Tyson *et al*., 2001). Three others cyclins (Cig1, 2 and Pug1) are lumped together; they play a secondary role at the start of the transition. The transition between the S/G_2 phase and the M-phase is described by the switch between metaphase promoting factor (MPF) and cdc13. The cell mass plays a crucial role. It doubles between birth and mitosis, after which it is divided by two. Moreover, the yeast cell may divide continuously, i.e. no special growth factors are needed.

Higher eukaryotes In mammals, regulatory mechanisms of phase transitions are different (Fig. 1). First of all, there are four distinct cdks and accordingly four different cyclin/cdk complexes that are activated one after the other. The transcription factor family of E2F/DP dimers (E2F1-6 with DP1, 2) and the pocket protein family (pRB, p107, p130) are central regulators of the mammalian cell cycle. E2F/DP regulate a large set of target genes including cyclin A, cyclin E, pRB, E2F1,2, DNA pol(α), PCNA and histone H2A. This underlines the relevance of the E2F/DP family for cell cycle transition.

Moreover, the tumor suppressor pRB plays an important role and was originally discovered in childhood cancer of the

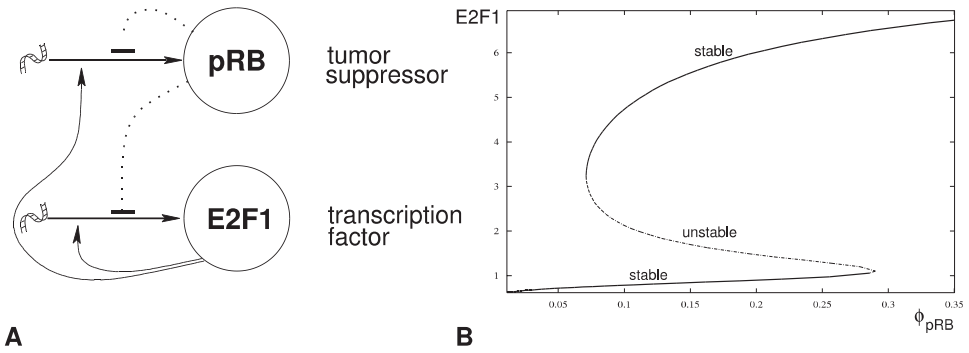

Fig. 2. The double inhibitor–activator module (**A**) shows bistability (**B**). In the bifurcation analysis, the degradation rate of retinoblastoma ϕ_{pRB} is used as the bifurcation parameter.

retina. It turned out to be the crucial inhibitor for the G_1/S phase progression. For these reasons, the pair E2F/DP and pRB can be regarded as the central player of the transition phenomena.

Another fundamental difference to the yeast cell cycle is the fact that both growth and proliferation depend strongly on mitogenic signals. A certain minimum duration and strength of signals are needed in order to induce the proliferation of resting cells.

DYNAMICS OF SMALL MODULES

It is widely believed that small units or modules can be regarded as key players in larger networks (termed also 'motifs' by other authors) (Chen *et al*., 2000; Milo *et al*., 2002; Tyson *et al*., 2001). Some key modules seem to be overrepresented in various networks. In other words, small motifs have a decision taking function. In order to exemplify this phenomenon, one can start with the analysis of such small units and try to enlarge them step-by-step. It will turn out that essential features, such as bistability, are robust with respect to modifications of the core module.

We start here with a well-known autocatalytic system. In this case a protein is its own transcriptional activator. This model applies to one of the most important protein complexes in the mammalian cells—the E2F/DP dimer. When we describe the auto-activation with certain non-linear functions (Iglesias and Levchenko, 2002), bistability can be observed. For instance, the following kinetic equation exhibits two stable steady states:

$$\frac{d}{dt}[\mathrm{E2F1}] = k\frac{a^2 + [\mathrm{E2F1}]^2}{K_{\mathrm{m}}^2 + [\mathrm{E2F1}]^2} - \phi[\mathrm{E2F1}],$$

where $a < K_{\mathrm{m}}$ and [E2F1] stands for the E2F/DP dimers. For a certain range of a bifurcation parameter, three steady states coexist, whereas outside this interval only a single steady state exists. This implies that slowly varying parameters can induce a sudden jump from low to high concentrations of the transcription factor E2F1. Interestingly, E2F1 is also a transcription factor for its inhibitor pRB. This tumor suppressor binds to the E2F/DP complex and causes inhibition of E2F1-induced transcription by masking its activation domain. The knock-down of pRB in HeLa cells (Whitfield *et al*., 2001) leads to an extremely fast proliferation. This underlines the central relevance of the E2F–pRB dynamics for cell cycle progression. Therefore, as a next step of the model development, we consider the coupling with pRB, a major control element at the G_1/S transition governed by phase specific pRB phosphorylation (Sherr, 1996). Since E2F1 is a transcription factor of pRB and E2F1 itself, we find here a double inhibition and a double activation. The following equations describe such a module (Fig. 2):

$$\frac{d}{dt}[\mathrm{pRB}] = k_1\frac{[\mathrm{E2F1}]}{K_{\mathrm{m1}} + [\mathrm{E2F1}]}\frac{J_{11}}{J_{11} + [\mathrm{pRB}]} - \phi_{\mathrm{pRB}}[\mathrm{pRB}]$$

$$\frac{d}{dt}[\mathrm{E2F1}] = k_p + k_2\frac{a^2 + [\mathrm{E2F1}]^2}{K_{m2}^2 + [\mathrm{E2F1}]^2}\frac{J_{12}}{J_{12} + [\mathrm{pRB}]} - \phi_{\mathrm{E2F1}}[\mathrm{E2F1}].$$

This module shows the desired bistability: E2F1 switches pRB off and jumps to a higher concentration. This double inhibitor–activator module constitutes the key element of the G_1/S network. As a bifurcation parameter we consider the degradation rate of pRB (Fig. 2). If either growth or proliferation signals are present in the cell environment, the expression of a 'growth factor sensor' cyclin D starts. The cyclin-dependent kinases cdk4, 6 as active subunits start to phosphorylate the tumor suppressor pRB. As mentioned above, the duration and strength of the mitogenic signal are crucial parameters in the stimulation phase. For a sufficient mitogenic signal, phosphorylation of pRB exceeds its dephosphorylation rate, and hence, the amount of a phosphorylated, less active pRB increases. The E2F/DP transcription complex is released and can activate its targets.

Consequently, we enlarge the pRB–E2F1 key module by cyclin D/cdk4, 6. Figure 1 (first extension) shows the double inhibitor–activator module with the phosphorylated form of pRB and cyclin D/cdk4, 6. The cell cycle is typically induced by mitogenic stimulation. The bifurcation analysis with the cyclin D/cdk4, 6 complex concentration as the bifurcation parameter shows bistability as well.

COUPLING TO THE GROWTH SIGNAL PATHWAY

The expression of cyclin D is the very end of the signaling cascades needed to conduct the growth signals from the extracellular space to the nucleus. First, an external signal has to be received by cell membrane receptors, which then further activate e.g. the Ras/Raf/MEK/ERK pathway (Chang *et al*., 2003). ERK activates a transcription factor, c-Jun, that forms complexes with Fos-proteins, called AP-1. In this way transcription factor AP-1 induces the G_1/S transition. We are now able to extend the model and take the strength of the mitogenic stimulation, F_m, as a bifurcation parameter. Next, we discuss the bifurcations due to the variation of F_m. Suppose the cell rests in the G_0 phase. Until a critical value, $F_{m_{crit}}$, of stimulation there should be no progress in the cell cycle as observed in experiments. In other words, we expect an existence of a threshold value for mitogenic growth and proliferation signals.

This is indeed what we get from the simulation (Fig. 3). A transcritical bifurcation is observed around $F_m = 0.0057$. The stable steady state loses its stability, and an unstable one becomes stable. This kind of bifurcation is commonly used to describe threshold phenomena. The definition of a restriction point between mid and late G_1 as the 'point of no return' is related to the described situation. A further increase in the stimulation parameter, F_m, leads to a saddle node bifurcation around $F_m = 0.0074$. This saddle node bifurcation represents the G_1/S phase transition associated with a sudden jump of the E2F/DP complex concentration. In Figure 4, we show the time course as an output from a simulation after a stepwise increase in F_m. One can easily recognize the switching behavior of the two antagonists E2F/DP and pRB.

SECOND EXTENSION AND FEEDBACKS

The first step in the inactivation of pRB is the phosphorylation of retinoblastoma by cyclin D/cdk4, 6 due to mitogenic signals. The E2F/DP related expression of cyclin E constitutes another positive-feedback loop. The kinase cdk2, activated by binding with cyclin E, is responsible for further phosphorylation of pRB. During the activation process an autocatalytic feedback via phosphorylation of the cyclin E/cdk2 inhibitor p27 is needed. This is implicitly considered in the present model, and the inhibitor p27 does not appear as a separate variable.

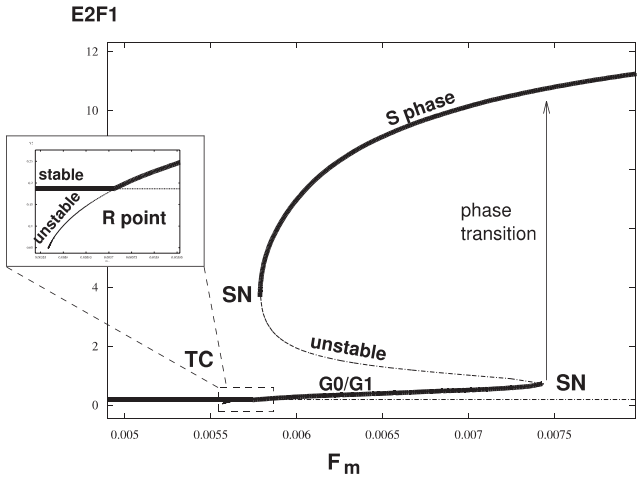

Fig. 3. Bifurcation diagram of G_1/S transition. Transcritical and saddle node bifurcations are shown. The strength of the mitogenic stimulation F_m is the bifurcation parameter.

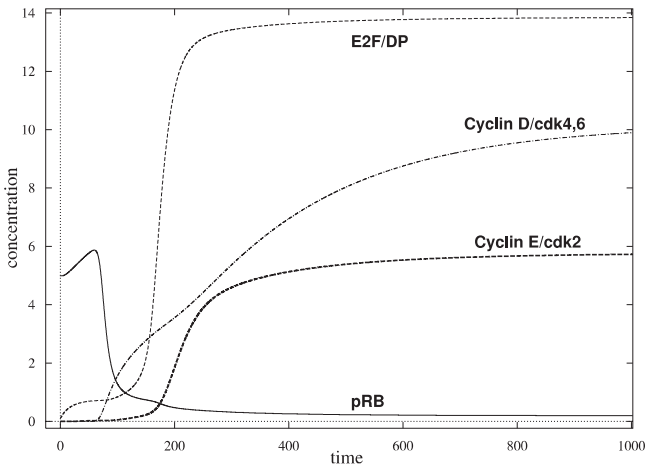

Fig. 4. Time course of computed protein concentrations. A strong mitogenic stimulus was used as an initial condition.

We again apply the bifurcation analysis and get the same dynamics as in Figure 3. The qualitative behavior of the system remains the same.

Finally, the influence of particular positive-feedbacks will be discussed. First, we assume different values for the constant K_{m4}, which expresses variable kinetics of the autocatalytic feedback loop of cyclin D/cdk4, 6. For decreasing K_{m4}, representing an enlargement of the positive feedback, the bifurcation thresholds are reduced drastically. This example illustrates that positive feedbacks can control cell proliferation effectively.

As discussed by Kel *et al*. (2000), E2F1-binding sites are present in the promoter of AP-1. This regulation constitutes another positive feedback. Interestingly, the transcritical

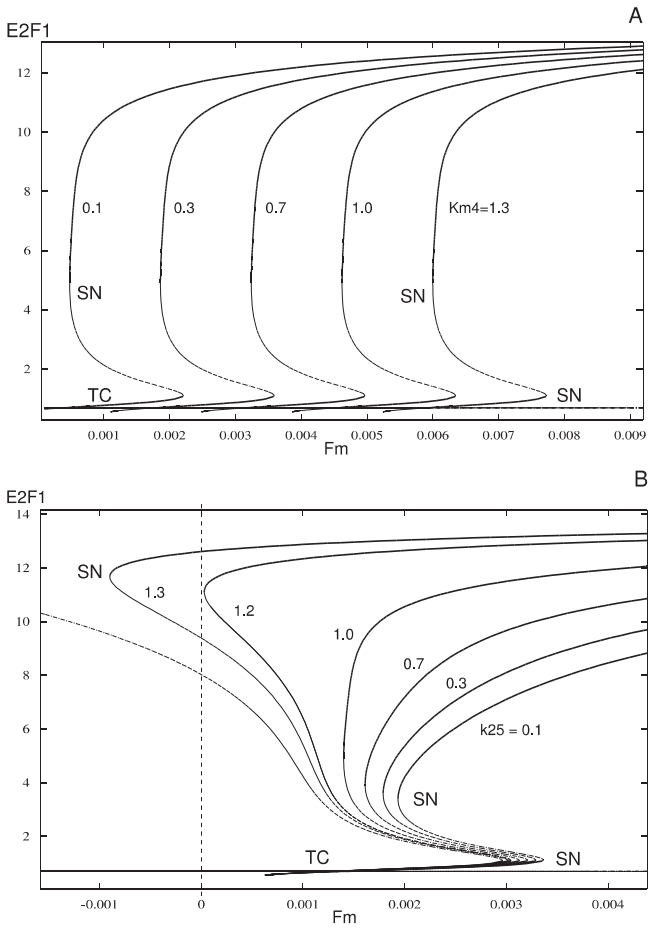

Fig. 5. Bifurcation diagrams illustrating the role of the feedbacks via cyclin D auto-activation (**A**) and AP-1 stimulation via E2F1 (**B**).

bifurcation is not influenced by this feedback (Fig. 5, lower graph). However, the typical S-shape of the bifurcation branch becomes more pronounced. For a strong positive feedback, even for a zero stimulation, two stable steady states exist. This implies irreversibility of the G_1/S transition: if E2F1 has once reached a high concentration, a removal of the mitogenic signal cannot lead to a return to the G_1 phase.

CONCLUSIONS

We developed a mathematical model describing the G_1/S transition of mammalian cells. We show that threshold phenomenon (restriction point R) and the G_1/S transition can be traced back to core modules. The double activator–inhibitor module of the antagonistic players E2F/DP and pRB make up the key unit of this phase transition. It turns out that the dynamics found in this basic system remains preserved in enlarged systems as well. This leads to the conclusion that some crucial elements in a network have a decision taking function. The second main result is a characterization of specific points of the cell cycle. The G_1-phase restriction point, R, is associated with a transcritical bifurcation commonly used to describe threshold phenomena. The G_1/S transition point is in turn described by a saddle-node bifurcation leading to bistability.

Our model is a very first step for representing common knowledge of the physiological mechanisms as a mathematical model. Even though most of the parameters are not available yet, basic phenomena such as the described bifurcations are robust features of positive feedback loops (Aguda, 2001; Blüthgen and Herzel, 2001; Tyson *et al*., 2001). Bifurcation theory can help identify basic modules in large networks even if kinetic parameters are missing.

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APPENDIX

Note that positive and negative feedbacks as indicated in Figure 1 are modeled by bilinear terms and Michaelis–Menten-like kinetics. Exponential degradation of all components is assumed. The full set of differential equations reads:

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{pRB}] = k_1 \frac{[\mathrm{E2F1}]}{K_{\mathrm{m1}} + [\mathrm{E2F1}]} \frac{J_{11}}{J_{11} + [\mathrm{pRB}]} \frac{J_{61}}{J_{61} + [\mathrm{pRB}_p]} - k_{16}[\mathrm{pRB}][\mathrm{CycD}_a] + k_{61}[\mathrm{pRB}_p] - \phi_{\mathrm{pRB}}[\mathrm{pRB}].$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{E2F1}] = k_p + k_2 \frac{a^2 + [\mathrm{E2F1}]^2}{K_{\mathrm{m2}}^2 + [\mathrm{E2F1}]^2} \frac{J_{12}}{J_{12} + [\mathrm{pRB}]} \times \frac{J_{62}}{J_{62} + [\mathrm{pRB}_p]} - \phi_{\mathrm{E2F1}}[\mathrm{E2F1}].$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{CycD}_i] = k_3[\mathrm{AP\text{-}1}] + k_{23}[\mathrm{E2F1}] \frac{J_{13}}{J_{13} + [\mathrm{pRB}]} \times \frac{J_{63}}{J_{63} + [\mathrm{pRB}_p]} + k_{43}[\mathrm{CycD}_a] - k_{34}[\mathrm{CycD}_i] \frac{[\mathrm{CycD}_a]}{K_{\mathrm{m4}} + [\mathrm{CycD}_a]} - \phi_{\mathrm{CycD}_i}[\mathrm{CycD}_i].$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{CycD}_a] = k_{34}[\mathrm{CycD}_i] \frac{[\mathrm{CycD}_a]}{K_{\mathrm{m4}} + [\mathrm{CycD}_a]} - k_{43}[\mathrm{CycD}_a] - \phi_{\mathrm{CycD}_a}[\mathrm{CycD}_a].$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{AP\text{-}1}] = F_{\mathrm{m}} + k_{25}[\mathrm{E2F1}] \frac{J_{15}}{J_{15} + [\mathrm{pRB}]} \frac{J_{65}}{J_{65} + [\mathrm{pRB}_p]} - \phi_{\mathrm{AP\text{-}1}}[\mathrm{AP\text{-}1}].$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{pRB}_p] = k_{16}[\mathrm{pRB}][\mathrm{CycD}_a] - k_{61}[\mathrm{pRB}_p] - k_{67}[\mathrm{pRB}_p][\mathrm{CycE}_a] + k_{76}[\mathrm{pRB}_{pp}] - \phi_{\mathrm{pRB}_p}[\mathrm{pRB}_p].$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{pRB}_{pp}] = k_{67}[\mathrm{pRB}_p][\mathrm{CycE}_a] - k_{76}[\mathrm{pRB}_{pp}] - \phi_{\mathrm{pRB}_{pp}}[\mathrm{pRB}_{pp}].$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{CycE}_i] = k_{28}[\mathrm{E2F1}] \frac{J_{18}}{J_{18} + [\mathrm{pRB}]} \frac{J_{68}}{J_{68} + [\mathrm{pRB}_p]} + k_{98}[\mathrm{CycE}_a] - k_{89}[\mathrm{CycE}_i] \frac{[\mathrm{CycE}_a]}{K_{\mathrm{m9}} + [\mathrm{CycE}_a]} - \phi_{\mathrm{CycE}_i}[\mathrm{CycE}_i].$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{CycE}_a] = k_{89}[\mathrm{CycE}_i] \frac{[\mathrm{CycE}_a]}{K_{\mathrm{m9}} + [\mathrm{CycE}_a]} - k_{98}[\mathrm{CycE}_a] - \phi_{\mathrm{CycE}_a}[\mathrm{CycE}_a].$$

The following parameters are chosen to reflect the experimentally known features:

$k_1 = 1,\ k_2 = 1.6,\ k_3 = 0.05,\ k_{16} = 0.4,\ k_{34} = 0.04,\ k_{43} = 0.01$

$k_{61} = 0.3,\ k_{67} = 0.7,\ k_{76} = 0.1,\ k_{23} = 0.3,\ k_{25} = 0.9,\ k_{28} = 0.06$

$k_{89} = 0.07,\ k_{98} = 0.01,\ a = 0.04$

$J_{11} = 0.5,\ J_{12} = 5,\ J_{15} = 0.001,\ J_{18} = 0.6, J_{61} = 5,\ J_{62} = 8$

$J_{65} = 6,\ J_{68} = 7,\ J_{13} = 0.002,\ J_{63} = 2$

$K_{\mathrm{m1}} = 0.5, K_{\mathrm{m2}} = 4,\ K_{\mathrm{m4}} = 0.3,\ K_{\mathrm{m9}} = 0.005,\ k_p = 0.05$

$\phi_{\mathrm{pRB}} = 0.005,\ \phi_{\mathrm{E2F1}} = 0.1,\ \phi_{\mathrm{CycD}_i} = 0.023,\ \phi_{\mathrm{CycD}_a} = 0.03$

$\phi_{\mathrm{AP\text{-}1}} = 0.01,\ \phi_{\mathrm{pRB}_p} = 0.06,\ \phi_{\mathrm{pRB}_{pp}} = 0.04, \phi_{\mathrm{CycE}_i} = 0.06$

$\phi_{\mathrm{CycE}_i} = 0.05$