Mathematical modeling of fission yeast *Schizosaccharomyces pombe* cell cycle: exploring the role of multiple phosphatases

P. Anbumathi · Sharad Bhartiya · K. V. Venkatesh

Received: 22 October 2011 / Accepted: 24 November 2011 / Published online: 8 December 2011
© Springer Science+Business Media B.V. 2011

Abstract Cell cycle is the central process that regulates growth and division in all eukaryotes. Based on the environmental condition sensed, the cell lies in a resting phase G0 or proceeds through the cyclic cell division process ($G1 \rightarrow S \rightarrow G2 \rightarrow M$). These series of events and phase transitions are governed mainly by the highly conserved Cyclin dependent kinases (Cdks) and its positive and negative regulators. The cell cycle regulation of fission yeast *Schizosaccharomyces pombe* is modeled in this study. The study exploits a detailed molecular interaction map compiled based on the published model and experimental data. There are accumulating evidences about the prominent regulatory role of specific phosphatases in cell cycle regulations. The current study emphasizes the possible role of multiple phosphatases that governs the cell cycle regulation in fission yeast *S. pombe*. The ability of the model to reproduce the reported regulatory profile for the wild-type and various mutants was verified through simulations.

Keywords Cell cycle · Biochemical kinetics based modeling · Fission yeast · Cell signaling · Phosphatases

Abbreviations

WT	Wild type
Cdc	Cell division cycle
Cdk	Cyclin dependent kinase
MPF	M phase promoting factor
preMPF	Inactive MPF

Introduction

Cell cycle is a crucial process during which an organism replicates all of its components and divides equally into two daughter cells (Nurse 2000). Traditionally the eukaryotic cell cycle is divided into four sequential phases namely, G1, S, G2 and M, each distinguished by specific events. Chromosome duplication occurs in the S phase while the duplicated chromosomes are equally segregated between the daughter cells in the M phase. Growth occurs during the G1 and G2 gap phases (Alberts et al. 2007). A primary motivation in gaining deeper insights into the molecular basis for cell cycle regulation and the quantification thereof lies in the fact that proliferative diseases such as cancer owe their origin to malfunctions in the cell cycle regulatory apparatus.

The cell cycle is driven primarily by the enzymatic activity of cyclin dependent kinases (Cdks) and its activation partner cyclin which is universally conserved across eukaryotes (Nurse 1990). Additional regulations are exerted by several activators and inhibitors through interlinked feedback loops between the regulators. Information related to the interaction of individual regulators is available through experimental studies and recent times are witnessing the overflow of high throughput experimental data (Rustici and Bahler 2001; Rustici et al. 2004; Peng et al. 2005; Oliva et al. 2005). Therefore, systematic modeling

Electronic supplementary material The online version of this article (doi:10.1007/s11693-011-9090-7) contains supplementary material, which is available to authorized users.

P. Anbumathi · S. Bhartiya (✉) · K. V. Venkatesh (✉)
Department of Chemical Engineering, Indian Institute of Technology Bombay, Mumbai 400 076, India
e-mail: bhartiya@che.iitb.ac.in

K. V. Venkatesh
e-mail: venks@che.iitb.ac.in

P. Anbumathi
e-mail: anbumathi_p@iitb.ac.in

approaches that explain the relevance of the underlying biochemical interactions are necessary to better understand the working of the cell cycle network (Nurse 2003). There are several review articles that discuss how combinations of these biochemical networks results in diverse biochemical, physiological characteristics (Ferrell 1996; Tyson et al. 1996, 2003; Alon 2007; Novak and Tyson 2008; Csikasz-Nagy et al. 2008b; Novak et al. 2010; Tyson and Novak 2010).

Various mathematical models that attempt to quantify the molecular mechanisms in cell cycle for different organisms have been reported in literature including those for *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Xenopus* Egg, *Caulobacter crescentus* and Mammalian system (Goldbeter 1991; Norel and Agur 1991; Aguda and Tang 1999; Aguda 1999; Gardner et al. 1998; Hatzimaniatis et al. 1999; Obeyesekere et al. 1999; Qu et al. 2003; Srividhya and Gopinathan 2006; Chauhan et al. 2008; Davidich and Bornholdt 2008; Faure et al. 2009; Iwamoto et al. 2011; Vasireddy and Biswas 2004). Pioneering works of Novak and Tyson's research group have established a strong foundation to mathematically model and analyze the cell cycle regulation of diverse model organisms (Tyson 1991; Novak and Tyson 1993, 1995, 1997; Marlovits et al. 1998; Novak et al. 1999, 2001; Ciliberto and Tyson 2000; Sveiczer et al. 2000; Chen et al. 2000, 2004; Tyson and Novak 2001, 2011; Ciliberto et al. 2003, 2005; Lovrics et al. 2006; Csikasz-Nagy et al. 2006, 2007; Toth et al. 2007; Li et al. 2009; Zwolak et al. 2009; Barik et al. 2010; Ball et al. 2011; Vinod et al. 2011). Their models have also successfully simulated several mutants, uncovered several governing principles and predicted several testable hypothesis, thus providing insights into the regulatory mechanisms.

Numerous extensive reviews describe the state of the art of modeling techniques employed to mathematically model the cell cycle control and regulation (Tyson et al. 2001, 2002, 2008; Ingolia and Murray 2004; Fuss et al. 2005; Sible and Tyson 2007; Tyson and Novak 2008; Csikasz-Nagy 2009; Novak et al. 2010). Comprehensive reviews that describe the physiological characteristics and the molecular regulations of fission yeast cell cycle regulation and control can also be found (Nasmyth 2001; Hayles and Nurse 1989; Nurse 1991, 2000, 2002; MacNeil and Nuse 1997; Moser and Russell 2000; Rupes 2002; Bahler 2005).

The motivation of the current study is to mathematically model the signaling networks that govern the cell cycle of fission yeast. It is known that fission yeast has only one Cdk Cdc2 which along with the B type cyclin Cdc13 that regulates cell cycle. Varying activity levels of this complex regulates the cell cycle at different stages of the cell cycle. In the G1 phase Rum1, Ste9 negatively regulates Cdc2-Cdc13. In S, G2 phase the Mik1, Wee1 negatively

regulates Cdc2-Cdc13 and Cdc25 positively regulates which promotes the G2-M transition. Completion of M specific events activates Slp1 which down regulates the Cdc2-Cdc13 activity through ubiquitin proteasome mediated degradation (Sveiczer et al. 2000; Novak et al. 2001; Csikasz-Nagy et al. 2006). The present study incorporates a few additional regulators that are involved in regulation of the M and M-G1 transition which are not considered in the previous models. Sveiczer et al. (2000), Novak et al. (2001) and Csikasz-Nagy et al. (2006) are the latest research articles that describes fission yeast cell cycle as a whole found in literature and the more recent publications describe specific events of the cell cycle rather than as a whole (Csikasz-Nagy et al. 2007, 2008a; Li et al. 2010). Therefore the current study attempts to build a mathematical model with more known regulations and the current study's interest also includes demonstrating the possible role of additional phosphatases in fission yeast cell cycle regulation. Phosphatases are known to play many regulatory roles during the cell cycle (Labib and Nurse 1993; Pallen et al. 1992; Kinoshita et al. 1990, 1993; Shimanuki et al. 1993). The prominent role of specific phosphatases in cell cycle regulation is appreciated only recently (Trinkle-Mulcahy and Lamond 2006; Queralt et al. 2006; Queralt and Uhlmann 2008; De Wulf et al. 2009). The current study emphasizes the possible role of multiple phosphatases that governs the cell cycle regulation in fission yeast *S. pombe*. We present here a dynamic model for fission yeast cell cycle with known and hypothesized molecular interactions that orchestrates the cell cycle regulation of fission yeast *S. pombe*. The goal here is to demonstrate the possible molecular architecture that could govern the cell cycle in fission yeast. The interlinked network is quantified by representing all enzymatic reactions by Michaelis-Menten kinetics, complex formations through mass action kinetics and by incorporating synthesis and degradation rates for all regulators. The model was used to simulate various mutants.

Cell cycle molecular map

The proposed model is based on the molecular interaction map presented in Sveiczer et al. (2000), Novak et al. (2001), and extending it by incorporating two hypotheses namely (1) use of two distinct phosphatases; and (2) regulation of one of the phosphatases through Securin-Separase sub-network. The cyclic behavior in cellular division is orchestrated by a network consisting of varying regulatory mechanisms including allosteric interactions and enzyme mediated phosphorylation/dephosphorylation cycles. Further, the phosphorylated and dephosphorylated proteins can exert a modulation effect on other interactions

active Ste9 (APCp-Ste9) are the two negative regulators of G1 phase (Labib et al. 1995; Kominami et al. 1998), which down regulate the M phase specific Cdc2-Cdc13 complex, also known as the Mitosis Promoting Factor (MPF), to ensure appropriate cell size at division. While Rum1 forms a complex with active Cdc2-Cdc13 for its proteolysis of the latter (CorreaBordes et al. 1997; Labib and Moreno 1996), APCp-Ste9 ubiquitinates Cdc13, Cdc2-Cdc13 and marks it for destruction (Blanco et al. 2000) until the cell grows and reaches a critical size. Eventually, the buildup of the G1 phase specific Cdk-cyclin complex namely, Cdc2-Puc1 and the S phase specific Cdk-cyclin complex namely Cdc2-Cig2, inhibits APCp-Ste9, characterizing the end of G1 phase (Sveiczer et al. 2000). The concomitant graded accumulation of MPF during late G1 phase is sufficient to inactivate Rum1 by phosphorylating it in the monomer as well in the trimer form namely, MPF-Rum1

[illegible]

(Sveicz et al. 2000; Benito et al. 1998). Ste9 is also kept in its inactive form (Ste9P) through Cdk2 (Puc1-cdc2; Cig2-Cdc2 and MPF) mediated phosphorylation during the other phases (Blanco et al. 2000).

The level of Cdc2-Cig2, also known as S-Phase Promoting Factor (SPF), is critical in activating DNA polymerase for replication firing (Stern and Nurse 1996). The antagonistic interaction between Cdc2-Cig and Rum1 also contributes in regulating the timing of S phase initiation (Benito et al. 1997; Novak and Tyson 1997). The S-phase and the subsequent G2 phase are regulated by two negative regulatory kinases namely Mik1 and Wee1, which keep MPF concentration low by phosphorylating it at Tyr 15 (Lundgren et al. 1991; Lee et al. 1994; Aligue et al. 1997; Kellogg 2003). The phosphorylated form of MPF, also known as preMPF, accumulates during S and G2 phases but possesses a very low kinase activity. Following Sveicz et al. (2000), we have assumed that the inactive preMPF contributes 5% towards the total kinase activity. Towards the end of the S phase, the quantity of MPF accrued is sufficient to down-regulate SPF, and the cycle progresses to the subsequent G2 phase of the cell cycle.

Fission yeast has an extended G2 phase wherein the gradual increase in the MPF activity positively regulates itself by promoting the activation of a phosphatase Cdc25 and further, negatively regulates the inhibitory kinase, Wee1 and Mik1 (Aligue et al. 1997). During the G1, S and until mid G2 phase, Cdc25 is kept inactive by a hypothetical phosphatase, termed in this study as PP1 (not to be confused with type 1 phosphatase). At the end of G2, active Cdc25 dephosphorylates the accumulated preMPF into active MPF whose level, therefore, rises sharply, marking the transition into the M-phase. In the M-phase, MPF activates its negative regulator Anaphase Promoting Factor (APC) through phosphorylation at multiple sites (Yamashita et al. 1999; Yamada et al. 2000). Experiments have suggested that a component of APC namely, Cut9 is regulated by a type 1 phosphatase (Yanagida 1998). Based on the above observation, we have assumed that APC is dephosphorylated by PP1 (see Fig. 1). The hyper-phosphorylated APC (APCp) forms an active complex APCp-Slp1 that marks all forms of cyclins for proteolysis including MPF, thus marking the end of M phase. APCp-Slp1 also promotes activation of Separase, a protease that degrades cohesin rings attached to the sister chromatids by degrading its inhibitor SecurinP (Funabiki et al. 1996a, b, 1997; Zachariae and Nasmyth 1999). During G1, S and G2 phases, the weak activity of MPF is sufficient to keep Separase in its inactive form by binding to SecurinP (Funabiki et al. 1996b; Kumada et al. 1998). Upon activation of Separase, it activates a hypothetical phosphatase termed PP2 in this study (not to be confused with type 2 phosphatase), which in turn activates Ste9. Active Ste9 replaces Slp1 in APCp and forms the

complex APCp-Ste9. This marks the transition from M phase at the end of cell division into the G1 phase of the subsequent cell cycle. In the absence of MPF mediated inhibition at the end of M phase, PP1 gets activated by auto-dephosphorylation (Wolfe and Gould 2004; Wolfe et al. 2006), which in turn activates all negative regulators and inactivates Cdc25 through enzymatic dephosphorylation (Trautmann et al. 2001; Wolfe and Gould 2004). Among the above interactions, the following interactions have been quantified for the first time (1) use two phosphatases; (2) a detailed molecular mechanism that drives the late M phase and early G1 phase events consisting of APC activation, Ste9 substitution of Slp1; (3) Securin-Separase sub-network; and (4) synthesis and degradation of all components. The molecular interactions used in the proposed model are consistent with those in other eukaryotic model organisms (Zachariae and Nasmyth 1999; Harper et al. 2002; Mendenhall and Hodge 1998; Kohn 1999). The molecular interactions were quantified using ordinary differential equations. The model parameter values were chosen to ensure that the model predictions for the various protein concentrations conform to the phase specific behavior reported in the literature.

Methods

A dynamic mathematical model consisting of ordinary differential equations (ODEs) was formulated to quantify the molecular interaction map shown in Fig. 1. Mass action kinetics was used for stoichiometric interactions and intermediate complex formations, while Michaelis–Menten kinetics was employed to represent enzyme mediated modification. It is appropriate to use ODEs because molecular diffusion, transcription, translation and membrane transport seem to be fast (within seconds) compared to the duration of cell cycle (hours; Tyson et al. 2001). The following represents the general form of the ordinary differential equation for conservation of mass for any component X,

$$\frac{d[x]}{dt} = S_x - k_{d_x}[x] \pm (k_{b_x} + k_{e_x}[E]([x]/K_{m_x} + [x])) \pm k_{MI}[MI] \quad (1)$$

where,

$[x]$ —concentration of protein X; S_x —rate of synthesis of protein 'x'; k_{d_x} —rate of degradation; k_{b_x} —rate of synthesis at basal level; k_{e_x} —rate of synthesis in the presence of enzyme; K_{m_x} —half saturation constant; $[E]$ —concentration of enzyme E; MI —formation or dissociation of complex; k_{MI} —rate of complex formation or dissociation.

The model consists of 35 differential equations, one algebraic equation and 137 parameters which are documented in supplementary information. In addition to the various proteins, the model also represents the change in the cell mass, which is assumed to grow at a constant growth rate. The parameter values were chosen to reflect the phase specific behavior and dynamics of the wild-type. We have adopted the criterion that the cell divides into two daughter cells when the total kinase activity of MPF falls below 0.1.

Results

Simulation of fission yeast cell cycle

The model using the parameters listed in Supplementary file 2 was simulated to obtain the dynamic profiles of various proteins regulating the fission yeast cell cycle. The simulation results capture the cyclic behavior of the various

regulatory proteins with a cell cycle period of about 150 min. The simulation results show the sequential activation and inactivation of various regulators that orchestrate proper cell division cycle.

G1-S-G2 regulation

In G1 and G2 phase of fission yeast cell growth is regulated and during S phase the genetic material is duplicated. Figure 2 presents the levels of the various regulators during the cell cycle. The G1 phase is marked by the activity of its regulators Rum1 (Fig. 2a) and APCp-Ste9 (Fig. 2d) as well as the buildup of G1 phase cyclin-Cdk complex, Cdc2- Puc1, and the S phase cyclin-Cdk complex, Cdc2-Cig2 (SPF). In this study the transition from G1 to S phase, which occurs approximately at 25–30 min, is marked by sufficient build up in the total kinase activity and the concomitant high SPF level (Zarzov et al. 2002). At this time, the G1 phase regulators, Rum1 and APCp-Ste9, exhibit a decreasing trend in their respective concentrations. Subsequent to the G1/S

Fig. 2 Simulation of wild type fission yeast *S. pombe* cell cycle regulation. The dynamics profiles of sequentially activated and inactivated regulators are seen over a period of two cycles and the cell cycle time is about 150 min. All the regulatory protein concentrations are normalized with their respective maximum concentrations. **a** G1 and S phase regulators Cdc2-Puc1 (solid), Cdc2-Cig2

(dash-dotted) and Rum1 (dotted); **b** Negative regulatory kinase Wee1 (dash-dotted), Mik1 (solid) and the positive regulator Cdc25p (dotted); **c** PP1 (dash-dotted), preMPF (dotted) and MPF (solid); **d** Mitotic exit and M-G1 transition regulators APCp-Slp1 (dash-dotted), Separase (solid), APCp-Ste9 (dashed), PP2 (dotted) and Mass

transition, at approximately 45 min, the total MPF kinase activity rises above a certain threshold while the SPF level drops to its basal values, marking the end of S phase in the present study. During the S phase, complete degradation of the G1 regulators, Rum1 and APCp-Ste9, as well as the G1 Cdk-cyclin complex, Cdc2-Puc1, is observed. Thus, the cell spends approximately 25–30 min in G1 phase and about 15–20 min in S phase. Subsequently, the cell progresses into the G2 phase, during which the S and G2 regulators namely, Mik1 and Wee1, get downregulated while the M phase specific positive regulator, Cdc25p, is upregulated (see Fig. 2b). The G2 phase approximately lasts for about 85–90 min. The antagonistic protein kinases and phosphatase activity here acts as amplifiers and as a switch and promotes G2-M transition (Nurse 2003).

M phase regulation, mitotic exit and M-G1 transition

Distribution of the duplicated chromosome and physical separation of the daughter cells are major events that occur during M phase and mitotic exit. The beginning of M phase is marked by a sharp increase in the MPF level and a corresponding decline in the preMPF level (Fig. 2c). The M phase also sees the upregulation of the negative regulator of the cyclins namely, APCp-Slp1 (Fig. 2d). The M phase, which lasts approximately 10–15 min, terminates with the degradation of all cyclins as well as the cyclin-Cdk complexes including MPF. The end of M phase marks the cleavage of the mother cell into two daughter cells. The transition from M to G1 phase is characterized by the sequential activation of APCp-Ste9, which is mediated indirectly by Separase (Fig. 2d) and also by the increase in the level of the phosphatase PP1 (Fig. 2d). The transition is also marked by the downregulation of Cdc25P and upregulation of all negative regulators namely, Mik1, Wee1, Rum1 and APCp-Ste9. It should be noted that although the Cdc2-Cdc13 complex builds throughout the cycle, it is kept in its inactive form namely, preMPF, until the end of G2 phase by the negative regulators. Figure 2d also depicts the increase in the cell mass and its division at the end of the cell cycle. The model demonstrates the role of Clp1 in both the initiation of mitosis and mitotic exit and by doing so, realizes the experimental observation that Clp1 could regulate part of the mechanism that coordinates two consecutive cell-cycle events (Fankhauser and Simanis 1993). One of the key insights that emerge from this model is that the possible role of a downstream phosphatase (the hypothesized PP2) under APCp-Slp1 regulation might play an important role during mitotic exit and M-G1 transition. The possible role of type 1 phosphatase in spindle assembly check point in fission yeast is known from experimental studies (Vanoosthuyse and Hardwick 2009). This is comparable to the phosphatase PP2 hypothesized in the present

model which could also play a prominent role in spindle assembly checkpoint.

Role of molecular mechanisms

As discussed previously, the current model incorporates detailed molecular mechanisms such as use of two phosphatases and the Securin-Separase sub-network. It is, therefore, of interest to identify their relevance in cell cycle regulation. To establish the role of a specific molecular interaction, we compared the wild type response with the response of a structurally perturbed network, which did not include the specific molecular interaction. Below, we discuss the role of a few of these interactions.

A unique feature of the current model is the use of a distinct Separase-Securin sub-network and PP2 activation through Separase. We eliminated the activation of PP2 by Separase as well as its inhibition by inhibitor I resulting in an unregulated PP2 level. Simulations showed that such an altered system exhibits a cyclic response with a marginally increased cell cycle time of 157 min (Fig. 3a). Here, PP2 is unregulated and thus available throughout the cell cycle ensuring availability of active Ste9 which, however, continues to be downregulated by MPF ensuring a cyclic behavior. Although in such a mutant, the concentration of Ste9 and PP2 exist at significantly higher levels than in the WT cell, the stoichiometric limitation and the inhibitory kinase activity of Cdc2-Puc1, Cdc2-Cig2 and MPF ensures that the APCp-Ste9 is similar to that observed in the WT. Another perturbation that was explored consisted of studying the role of the Securin-Separase sub-network by eliminating the Securin-Separase subsystem and directly activating the phosphatase PP2 with MPF while leaving the parameters unchanged. Simulations show an inviable phenotype with high MPFa (dashed line in Fig. 3b) and low PP1 (dashed-dot in Fig. 3b) which inactivates Cdc25p and activates the other negative regulators during M-G1 transition (Fig. 3b). This indicates the importance of the delay that arises through the Securin-Separase sub network which provides the essential time for PP1 to accumulate and increase its own activity autocatalytically to down-regulate the MPF through Cdc25, Wee1 and Mik1. A strain with an MPF independent phosphorylation of PP1 was also studied using the model. Simulations indicate a loss of cyclic response (result not shown). An MPF independent phosphorylation implies that the phase specific information, as quantified by the threshold of MPF, is not reflected in the PP1 levels. The consequent high levels of the MPF independent PP1 ensure that the G2 phase regulators, Wee1 and Mik1, are active and the positive regulator of the cell cycle Cdc25 remain inhibited, resulting in the failure of the cell cycle to enter M-phase. This demonstrates the central role of PP1 for activation of the negative regulators in

Fig. 3 Simulation results indicating dynamic profile of the various regulators in the cell cycle regulation of structurally perturbed mutant strains. All the regulatory protein concentrations are normalized with their respective maximum concentrations. **a** In this simulation PP2 is not regulated by Separase activation or though its inhibitor (I). Unregulated PP2 (*dashed*) and its role in cell cycle regulation, which results in a slightly enhanced cell cycle time of 157 min. The availability of PP2 throughout the cell cycle alters free Ste9 levels (*dash-dotted line*). MPF (*solid*) and APCp-Ste9 (*dotted*) are also shown; **b** The activation of PP2 is redirected through MPF (*dashed*) instead of Securin-Separase sub-network which leads to inviable phenotype due to the loss of essential time for PP1 (*dotted-dash line*) activation during mitotic exit; **c** In this mutant strain, all

dephosphorylations in the cell cycle regulation are based on a single phosphatase namely, PP2, that is, the role of PP1 is replaced by PP2 which results in inviable phenotype. The low level of PP2 (*dotted*) is not able to activate the essential regulators for M-G1 transition and the MPF ($- + -$) activity remains high which also keeps PP1 that has no regulatory role in this strain at its low; **d** In this strain, all dephosphorylations in the cell cycle regulation are based on a single phosphatase namely, PP1, that is, the role of PP2 is replaced by PP1 which results in a viable phenotype with enhanced cycle time of 165 min due to delay in M-G1 transition related events. The individual regulatory profiles of the crucial regulators such as MPF (*solid*), PP1 (*dotted-dash line*), APCp-Slp1 (*dotted*) and APCp-Ste9 (*solid*) resembles that of wild type

addition to the deactivation of the positive regulators of MPF at the end of M-phase.

To delineate the role of PP1, PP1 was replaced with PP2 in all PP1-related interactions while keeping the same set of parameters. Simulations show that these results in an inviable phenotype (refer Fig. 3c). Since in our model, PP2 has lower activity than PP1, enhanced levels of APCp-Slp1 are observed which continuously maintains low MPF level and towards end of the first cycle the inability of very low level

of PP2 to up-regulate the negative regulators leads to inviability. On the other hand, if PP2 is used only for deactivation of APCp instead of PP1, while maintaining the role of PP1 intact for all other regulations, the simulation results in yet another inviable phenotype (results not shown). In this case, due to the fact that the weak activation of the APCp-Slp1 results in improper degradation of MPF and its level remains high the cell never proceeds through M-G1 transition. To study the role of PP2, PP2 was replaced with

PP1 for the activation of Ste9, while maintaining the same set of parameters. Model simulations show regulatory phenotype which closely resembles wild type cell except for the cell cycle time of 165 min (see Fig. 3d). In this case, the delayed onset of Ste9 activation by PP1 relative to PP2 results in the extended cell cycle duration. These results demonstrate that although a single negative regulatory phosphatase PP1 can ensure a cyclic behavior, the phase specific regulations are disturbed, which indicates the possible role of more than one phosphatase in time specific the regulation of cell cycle in fission yeast.

Model prediction for deletion mutants

The model can also be used to predict the experimentally reported behavior of numerous mutants obtained by disrupting the various components of the cell cycle regulatory network. The proposed model could capture the various

single, double, temperature sensitive, over-expression and structural mutants. The deletion or knockout mutants were simulated by setting their synthesis rate constant to zero and in the case of other phenomenon like structural, temperature sensitive and over expression mutants the respective kinetic rate constants were decreased or increased (refer column 3 in Table S2 of supplementary file 3 which specifies the parameter(s) reset to simulate mutants). The supplementary information (supplementary file 3) shows the concentration profiles predicted by the model for a few interesting mutants. Below, we briefly discuss a few deletion mutants reported in literature and the corresponding model predictions.

The Wee1 Δ mutant has been studied experimentally (Fantes and Nurse 1978; Nurse and Thuriaux 1980) as well as through models (Sveiczzer et al. 2000; Novak et al. 2001). In the Wee1 Δ mutant (Fig. 4), the absence of the G2 regulator, Wee1, results in an early dephosphorylation of

Fig. 4 Prediction of cell cycle regulation of Wee1 Δ mutant of *S. pombe*. *S. Wee1* (Equation 15 in Supplementary file 1) and the initial condition for Wee1 (Equation 15 in Supplementary file 1) and Wee1P (Equation 16 in Supplementary file 1) differential equations are set to 0. The dynamic profiles of the various regulators of cell cycle are shown for two cycles. The cell cycle time reduced to 145 min when compared to WT and the G2 size control is also lost and the cell divides with half the size of WT fission yeast. All the regulatory protein

concentrations are normalized with their respective maximum concentrations. **a** G1- S regulators Cdc2-Puc1 (solid black), Cdc2-Cig2 (dash-dotted) and Rum1 (dotted); **b** Regulatory kinase Mik1 (solid black) and the positive regulator Cdc25p (dotted); **c** PP1 (dash-dotted), preMPF (dotted) and MPF (solid); **d** Mitotic exit and M-G1 transition regulators APCp-Slp1 (dash-dotted), Separase (solid), APCp-Ste9 (dashed), PP2 (dotted)

preMPF to MPF and consequently, the cell divides in about 145 min with approximately half the size of the WT cell. The model predictions are consistent with experimental observations by Nurse and Thuriaux (1980), which has shown that *Wee1* Δ cells enter mitosis at approximately half the size and exhibit a slight reduction in the doubling time. Simulations indicate that the total period for G1 and S phase is 75 min (see Fig. 4a), the G2 phase is shortened to 55 min which is due to the rapid accumulation of MPF in the absence of *Wee1* regulatory kinase. An elevated level of *Rum1* is observed until the 70th minute of the cell cycle (see Fig. 4a). Experimental studies do not show a significant difference in G1 or S phase duration of these mutants (Novak and Mitchison 1986) and thus support the simulation results. Thus, in the absence of *Wee1*, *Mik1* alone partially regulates *Cdc2*-*Cdc13* phosphorylation and the mutant cells are viable although with loss in G2 size control.

The model was also used to simulate the *Cdc25* Δ mutant, which is inviable in the absence of any other backup phosphatases (Millar et al. 1992; see Fig. 5). The absence of the positive regulator *Cdc25p* that promotes G2-M transition results in the inability to dephosphorylate inactive preMPF

(see Fig. 4c dash-dotted line) to its active form, that is, MPF which is necessary to promote the cell into M phase. As a result, the cell gets blocked in G2, grows in size and eventually dies. It has been reported that fission yeast can withstand absence of mitotic inducer *Cdc25* under two circumstances, either in *cdc2-3w* or in *wee1* genetic background (Sveiczer et al. 1999). In both the mutants, the backup phosphatases *Pyp3*, *Pyp2* and *Pyp1* play a major role of reversing the *Mik1* mediated Tyr 15 phosphorylation of *Cdc2*-*Cdc13* that limits G2-M transition (Millar et al. 1992). One of the mutants with *Cdc25* Δ background, *Wee1*-*Cdc25* Δ shows cell cycles of varying cycle time (Sveiczer et al. 2000). In the simulation to mimic the role of the weak phosphatases of the *Pyp* family in absence of *Cdc25*, we used a low activity of *Cdc25p* in the place of *Pyp* phosphatases. The model demonstrates varying cell division cycles which alternate with cell cycle times of 170 and 190 min for about four cycles (results not shown). It has been reported that the *Wee1*-*Cdc25* Δ mutant strain shows a quantized cell cycle with a distinct cycle periods, which is not reflected by the current model (Sveiczer et al. 2000). Thus, the model can simulate and reflect several mutants

Fig. 5 Prediction of cell cycle regulation of *Cdc25* Δ mutant of *S. pombe*. *S_Cdc25* (Equation 17 in Supplementary file 1), *k_a_pM* (Equation 36 in Supplementary file 1) and the initial condition for *Cdc25* (Equation 17 in Supplementary file 1), *Cdc25P* (Equation 18 in Supplementary file 1) differential equations are set to 0. Absence of positive regulator of M phase *Cdc25* results in inviable phenotype with MPF accumulated in its phosphorylated inactive state preMPF.

a G1 and S phase regulators *Cdc2*-*Puc1* (solid), *Cdc2*-*Cig2* (dash-dotted) and *Rum1* (dotted); **b** Negative regulatory kinase *Wee1* (dash-dotted), *Mik1* (solid black) and the positive regulator *Cdc25p* (dotted); **c** *PP1* (dash-dotted), preMPF (dotted) and MPF (solid); **d** Mitotic exit and M-G1 transition regulators *APCp*-*Slp1* (dash-dotted), *Separase* (solid), *APCp*-*Ste9* (dashed), *PP2* (— + —) and *Mass* (solid)

Fig. 6 Prediction of cell cycle regulation of double mutant Rum1ΔSte9Δ mutant of *S. pombe*. S_Rum1, S_Ste9 and their respective initial condition for were set to 0. Absence of both the negative regulators of G1 phase results in viable cells with very short G1 phase and the approximate cycle time is 125 min. Note that the cell also divides with small size and the size keeps decreasing over

time **a** G1 and S phase regulators Cdc2-Puc1 (solid black), Cdc2-Cig2 (dash-dotted); **b** Negative regulatory kinase Wee1 (dash-dotted), Mik1 (solid black) and the positive regulator Cdc25p (dotted); **c** PP1 (dash-dotted), preMPF (dotted) and MPF (solid); **d** Mitotic exit and M-G1 transition regulators APCp-Slp1 (dash-dotted), Separase (solid), PP2 (dotted) and Mass

observed experimentally and the details of which are listed in Table S2 in supplementary file 3.

Another well characterized mutant simulated in this study is Rum1Δ Ste9Δ double mutant, which is known to be viable as shown in Fig. 6 (Kitamura et al. 1998; Yamaguchi et al. 2000). Due to the absence of the G1 phase regulators Ste9 and Rum1 the relative time spend in G1 phase is considerably less compared to wild type cells and there is no clear distinction between G1 and S phase (see Fig. 6). The simulation results indicate a short G1, S period of approximately 20–30 min and results in overall cycle time of approximately 125 min. Considerable activity of the Ste9 regulatory phosphatase PP2 (see Fig. 6c dotted line) is seen active till G2 phase in this mutant.

Discussion

Mitotic cell division cycle of all eukaryotes is regulated by interlinked feedback regulations that drive cells

dynamically for growth and cell division. While experiments have elicited these interactions piecemeal, mathematical modeling enables integrating the various experimental observations and provides insights into the operation of the cyclic process. The current study evaluated the hypothesized molecular interaction map shown in Fig. 1. Model simulations for wild type and mutants represent the experimental observation of several cell cycle regulators of fission yeast cell cycle. Switching behavior observed in biological regulatory networks is due to cooperative processes, positive feedback loop or enzyme saturation (Ferrell 1996). In the cell cycle network the above mentioned structures and functions plays critical role in carrying out the most crucial process ‘cell cycle’. The wild type model and the mutants predicted are in reasonable agreement with the experimental results available in literature (Table S2 in supplementary file 3).

Comparing to the previous models, the mitotic exit regulatory events in the present study were expanded by including Separase-Securin sub-network which plays a

crucial role in governing late M phase and M-G1 transition. The current model comprises of two distinct regulatory phosphatases and a detailed M-G1 transition which was able to demonstrate the cell cycle events of fission yeast *S. pombe*. Specifically, this work hypothesizes two distinct phosphatases, PP1 and PP2, wherein PP2 is considered to be Separase regulated and influences M-G1 transition. Both the phosphatases participate in controlling several regulators that aid in the termination of one cycle and initiating the next round of the cell cycle. The model also incorporates the synthesis and degradation of all components in the network. Thus, the cyclic response of the proposed model is purely a manifestation of the molecular interactions. It is well known that multiple phosphatases play a role in the cell cycle regulation of eukaryotes (De Wulf et al. 2009). Several earlier models (Sveicz et al. 2000) have used a single phosphatase (termed as PP or single intermediary enzyme IE) which was able to provide useful insights into the working of the cell cycle (Sveicz et al. 2000; Novak et al. 2001). In the current model, the APCp-Slp1 complex, which ubiquitinates all cyclins (free cdc2 bound) for degradation at the end of M phase, is partly regulated through negative self regulation and through APCp-Ste9 complex formation. This complex degrades only M phase specific cyclin, Cdc13, and its active complex MPF in the subsequent G1 phase. The time delay between the activation of APC and formation of APCp-Ste9, which is due to the Separase-Securin sub-network, corresponds to the crucial late M to early G1 phase transition.

The consideration of PP1 negatively regulating Cdc25 phosphatase and positive regulation of the negative regulators Rum1, Wee1 and Mik1 is inspired by experimental evidences that suggest the possible role of the Cdc14 family phosphatase, Clp1 at different stages of fission yeast cell cycle regulation (Trautmann et al. 2001; 2004; Wolfe and Gould 2004; Stegmeier and Amon 2004; Wolfe et al. 2006). Clp1 is shown to play diverse roles in fission yeast cell cycle regulation especially at mitotic exit (Fankhauser and Simanis 1993; Trautmann et al. 2001; Wolfe and Gould 2004). Clp1 is known to participate in a wide varieties of regulatory events including G2 arrest through Cdc25 inhibition, septation initiation and activation of negative regulator, Wee1 and inhibition of the positive regulator, Cdc25p, during mitotic exit (Trautmann et al. 2001; Wolfe and Gould 2004; Esteban et al. 2004). Recent studies have also shown the possible role of Clp1 during replication stress (Chen et al. 2008). Further, a boolean network model that faithfully demonstrates the sequence of regulatory proteins along the cell cycle of fission yeast, highlights the possible candidature of the only phosphatase PP used in their study, to be Clp1 (Davidich and Bornholdt 2008). Through experimental studies, it is known that Clp1 localizes in the nucleolus during the normal cell cycle

(Trautmann and McCollum 2002, 2005; Clifford et al. 2008a, b). However, the mechanisms by which it is transported from nucleolus to nucleus and cytoplasm are not clear (Chen et al. 2008). The auto-dephosphorylation mechanism for activation of Clp1 has been shown by Wolfe et al. (2006), which has also been adopted in the present study for activation of PP1. In our mutant prediction analysis, deletion of PP1 results in an inviable phenotype (refer Supplementary 3 Table S2) due to failure in downregulation of Cdc25p and activation of the negative regulators of the cell cycle. However, it is known through experimental studies that absence of Clp1 advances prematurely into mitosis because of the absence of the negative regulation of Cdc25p (Trautmann et al. 2001; Wolfe and Gould 2004). Therefore, a simulation was performed by decreasing the synthesis level of PP1 to 75% of its nominal value, which results in a viable phenotype with short cycle period of 125 min due to irregular control of Cdc25p and other negative regulators during mitotic exit (see Table S2, S. No. 9, in Supplementary File 3). This closely reflects the experimental observation of premature advancement of cell cycle (Trautmann et al. 2001; Wolfe and Gould 2004). This indicates that there could be more than one phosphatase that crucially regulates the cell cycle events. Thus, PP1 in this study represents the cumulative activity of several phosphatases that governs fission yeast cell cycle regulation.

The inclusion of hypothetical phosphatase PP2 in the current model is inspired by several experimental evidences that suggest the possible role of phosphatases in regulating mitotic exit (Labib and Nurse 1993; Pallen et al. 1992; Trinkle-Mulcahy and Lamond 2006; De Wulf et al. 2009; Vanoosthuyse and Hardwick 2009; Shimanuki et al. 1993). In budding yeast a phosphatase, Cdc14 is regulated indirectly through Separase during mitosis exit (Queralt et al. 2006). The above studies show the prominent regulatory role of specific phosphatases in cell cycle regulations. One of the key insights obtained from the model is that the possible role of a downstream phosphatase under APCp-Slp1 regulation which might play an important role during mitotic exit and M-G1 transition. The possible role of type 1 phosphatase in spindle assembly check point in fission yeast is shown experimentally (Vanoosthuyse and Hardwick 2009), which is comparable to phosphatase PP2 hypothesized in the current model and may also play a prominent role in spindle assembly checkpoint. The prominent role of specific phosphatases in cell cycle regulation is appreciated only recently. In fact, Queralt et al. (2006) have identified a downstream regulatory phosphatase based on a hypothetical phosphatase previously considered in a mathematical model (Chen et al. 2004). The current study speculates a possible downstream role for a phosphatase under APCp-Slp1 control, which, needs to be validated through experiments. Insights gained from such modeling studies can be utilized to design

experiments to further understanding the molecular role of diverse phosphatases in regulating fission yeast cell cycle.

The individual regulatory role of the Securin-Separase sub-network and phosphatases were analyzed through modification of the network architecture. In Fig. 3a when PP2 is not regulated by upstream Separase-Securin sub-network and its hypothetical inhibitor I, the simulation results show cell division with slightly enhanced cycle time of 157 min which indicates that the inter connected network of Separase—Securin sub-network mediated regulation of PP2 plays crucial role in deciding the timing of mitotic exit and in turn M-G1 transition. When the regulatory role of PP2 was replaced by PP1, simulation results show that single phosphatase PP1 alone can regulate the cell cycle regulation of fission yeast. This is similar to the results by Sveiczzer et al. (2000) and Novak et al. (2001). Here slightly enhanced cycle time of 165 min for cell division was observed and the phenotype was similar to that of WT (Fig. 3d). However on the contrary if PP1 was replaced with PP2 the cyclic response was disturbed and resulted in inviable phenotype (Fig. 3c). These analyses along with the PP1Δ mutant and the partial expression (75% of nominal synthesis rate) indicates that from a modeling standpoint, multiple phosphatases play a role in imparting phase specificity. However, ambiguities exist in the identity and roles of the different phosphatases in the regulation of the cell cycle from an experimental perspective.

In this study and in several early modeling studies (Novak et al. 1998; Sveiczzer et al. 2000; Csikasz-Nagy et al. 2006; Davidich and Bornholdt 2008; Li et al. 2010) an extended regulatory network is considered to regulate the cell cycle growth and division processes of fission yeast *S. pombe*. A more recent experiment by Coudrouse and Nurse (Coudrouse and Nurse 2010) demonstrates that orderly progression through the major events of the cell cycle can be driven by oscillation of an engineered monomolecular cyclin-dependent protein kinase (CDK) module lacking much of the canonical regulation. The primary organizer or regulator here is the Cdc2-Cdc13 oscillator and its activity thresholds that define the timing, direction of cell cycle and its independent events like DNA replication, mitosis and its orderly progression. A simple model by Tyson and Novak (Tyson and Novak 2011) demonstrates this phenomenon. The detailed modeling procedure adopted in the current study, however, may provide insights into the role of the individual regulator in governing the cell cycle regulatory events in wild type fission yeast *S. pombe*.

Conclusion

The current model comprised of two distinct regulatory phosphates and a detailed M-G1 transition which was able to

demonstrate the cell cycle events of fission yeast *S. pombe*. The molecular interaction map incorporated in the current study was able to predict the behavior of a few classical mutant phenotypes. The current modeling study highlights the role of multiple phosphates in the cell cycle regulation. However, the specific phosphatases and their respective roles need to be unraveled through experimental studies.

Acknowledgments PA acknowledges P K Vinod and Nikhil Chaudhary, for useful discussions. KVV acknowledges funds from Department of Science and Technology (DST), India.

References

- Aguda BD (1999) A quantitative analysis of the kinetics of the G(2) DNA damage checkpoint system. *Proc Natl Acad Sci USA* 96(20):11352–11357
- Aguda BD, Tang Y (1999) The kinetic origins of the restriction point in the mammalian cell cycle. *Cell Prolif* 32(5):321–335
- Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P (2007) *Molecular biology of the cell*, fifth edition. Garland Science Publishers, London
- Aligue R, Wu L, Russell P (1997) Regulation of *Schizosaccharomyces pombe* Wee1 tyrosine kinase. *J Biol Chem* 272(20):13320–13325
- Alon U (2007) Network motifs: theory and experimental approaches. *Nat Rev Genet* 8(6):450–461. doi:10.1038/Nrg2102
- Bahler J (2005) Cell-cycle control of gene expression in budding and fission yeast. *Annu Rev Genet* 39:69–94. doi:10.1146/annurev.genet.39.110304.095808
- Ball DA, Ahn TH, Wang PY, Chen KC, Cao Y, Tyson JJ, Peccoud J, Baumann WT (2011) Stochastic exit from mitosis in budding yeast model predictions and experimental observations. *Cell Cycle* 10(6):999–1009. doi:10.4161/cc.10.6.14966
- Barik D, Baumann WT, Paul MR, Novak B, Tyson JJ (2010) A model of yeast cell-cycle regulation based on multisite phosphorylation. *Mol Syst Biol* 6. doi:10.1038/Msb.2010.55
- Benito J, Martin C, Arellano M, Blanco M, Sanchez A, Bolanos P, Moreno S (1997) Regulation of progression through start in fission yeast. *Eur J Cell Biol* 72:2
- Benito J, Martin-Castellanos C, Moreno S (1998) Regulation of the G(1) phase of the cell cycle by periodic stabilization and degradation of the p25(rum1) CDK inhibitor. *EMBO J* 17(2):482–497
- Blanco MA, Sanchez-Diaz A, de Prada JM, Moreno S (2000) APC(ste9/srw1) promotes degradation of mitotic cyclins in G(1) and is inhibited by cdc2 phosphorylation. *EMBO J* 19(15):3945–3955
- Chauhan A, Legewie S, Westermarck PO, Lorenzen S, Herzel H (2008) Mesoscale model of G1/S phase transition in liver regeneration. *J Theor Biol* 252(3):465–473. doi:10.1016/j.jtbi.2008.01.020
- Chen KC, Csikasz-Nagy A, Gyorffy B, Val J, Novak B, Tyson JJ (2000) Kinetic analysis of a molecular model of the budding yeast cell cycle. *Mol Biol Cell* 11(1):369–391
- Chen KC, Calzone L, Csikasz-Nagy A, Cross FR, Novak B, Tyson JJ (2004) Integrative analysis of cell cycle control in budding yeast. *Mol Biol Cell* 15(8):3841–3862. doi:10.1091/mbc.E03-11-0794
- Chen CT, Feoktistova A, Chen JS, Shim YS, Clifford DM, Gould KL, McCollum D (2008) The SIN kinase Sid2 regulates cytoplasmic retention of the *S. pombe* Cdc14-like phosphatase Clp1. *Curr Biol* 18(20):1594–1599. doi:10.1016/j.cub.2008.08.067

- Ciliberto A, Tyson JJ (2000) Mathematical model for early development of the sea urchin embryo. *Bull Math Biol* 62(1):37–59
- Ciliberto A, Novak B, Tyson JJ (2003) Mathematical model of the morphogenesis checkpoint in budding yeast. *J Cell Biol* 163(6):1243–1254. doi:[10.1083/jcb.200306139](https://doi.org/10.1083/jcb.200306139)
- Ciliberto A, Lukacs A, Toth A, Tyson JJ, Novak B (2005) Rewiring the exit from mitosis. *Cell Cycle* 4(8):1107–1112
- Clifford DM, Chen CT, Roberts RH, Feoktistova A, Wolfe BA, Chen JS, McCollum D, Gould KL (2008a) The role of Cdc14 phosphatases in the control of cell division. *Biochem Soc Trans* 36:436–438. doi:[10.1042/Bst0360436](https://doi.org/10.1042/Bst0360436)
- Clifford DM, Wolfe BA, Roberts-Galbraith RH, McDonald WH, Yates JR, Gould KL (2008b) The Clp1/Cdc14 phosphatase contributes to the robustness of cytokinesis by association with anillin-related Mid1. *J Cell Biol* 181(1):79–88. doi:[10.1083/jcb.200709060](https://doi.org/10.1083/jcb.200709060)
- CorreaBordes J, Gulli MP, Nurse P (1997) p25(rum1) promotes proteolysis of the mitotic B-cyclin p56(cdc13) during G(1) of the fission yeast cell cycle. *EMBO J* 16(15):4657–4664
- Coudreuse D, Nurse P (2010) Driving the cell cycle with a minimal CDK control network. *Nature* 468(7327):1074–1474. doi:[10.1038/Nature09543](https://doi.org/10.1038/Nature09543)
- Csikasz-Nagy A (2009) Computational systems biology of the cell cycle. *Brief Bioinf* 10(4):424–434. doi:[10.1093/Bib/Bbp005](https://doi.org/10.1093/Bib/Bbp005)
- Csikasz-Nagy A, Battogtokh D, Chen KC, Novak B, Tyson JJ (2006) Analysis of a generic model of eukaryotic cell-cycle regulation. *Biophys J* 90(12):4361–4379. doi:[10.1529/biophysj.106.081240](https://doi.org/10.1529/biophysj.106.081240)
- Csikasz-Nagy A, Kapuy O, Gyorffy B, Tyson JJ, Novak B (2007) Modeling the septation initiation network (SIN) in fission yeast cells. *Curr Genet* 51(4):245–255. doi:[10.1007/s00294-007-0123-4](https://doi.org/10.1007/s00294-007-0123-4)
- Csikasz-Nagy A, Gyorffy B, Alt WG, Tyson JJ, Novak B (2008a) Spatial controls for growth zone formation during the fission yeast cell cycle. *Yeast* 25(1):59–69. doi:[10.1002/Yea.1571](https://doi.org/10.1002/Yea.1571)
- Csikasz-Nagy A, Novak B, Tyson JJ (2008b) Reverse engineering models of cell cycle regulation. *Cell Oscil Mech* 641:88–97
- Davidich MI, Bornholdt S (2008) Boolean network model predicts cell cycle sequence of fission yeast. *Plos One* 3(2). doi:[10.1371/Journal.Pone.0001672](https://doi.org/10.1371/Journal.Pone.0001672)
- De Wulf P, Montani F, Visintin R (2009) Protein phosphatases take the mitotic stage. *Curr Opin Cell Biol* 21(6):806–815. doi:[10.1016/j.ceb.2009.08.003](https://doi.org/10.1016/j.ceb.2009.08.003)
- Esteban V, Blanco M, Cueille N, Simanis V, Moreno S, Bueno A (2004) A role for the Cdc14-family phosphatase Fip1p at the end of the cell cycle in controlling the rapid degradation of the mitotic inducer Cdc25p in fission yeast. *J Cell Sci* 117(12):2461–2468. doi:[10.1242/Jcs.01107](https://doi.org/10.1242/Jcs.01107)
- Fankhauser C, Simanis V (1993) The *Schizosaccharomyces pombe* Cdc14 gene is required for septum formation and can also inhibit nuclear division. *Mol Biol Cell* 4(5):531–539
- Fantes PA, Nurse P (1978) Control of timing of cell-division in fission yeast—cell-size mutants reveal a 2nd control pathway. *Exp Cell Res* 115(2):317–329
- Faure A, Naldi A, Lopez F, Chaouiya C, Ciliberto A, Thieffry D (2009) Modular logical modelling of the budding yeast cell cycle. *Mol Biosyst* 5(12):1787–1796. doi:[10.1039/B910101m](https://doi.org/10.1039/B910101m)
- Ferrell JE (1996) Tripping the switch fantastic: how a protein kinase cascade can convert graded inputs into switch-like outputs. *Trends Biochem Sci* 21(12):460–466
- Funabiki H, Kumada K, Yanagida M (1996a) Fission yeast cut1 and cut2 are essential for sister chromatid separation, concentrate along the metaphase spindle and form large complexes. *EMBO J* 15(23):6617–6628
- Funabiki H, Yamano H, Kumada K, Nagao K, Hunt T, Yanagida M (1996b) Cut2 proteolysis required for sister-chromatid separation in fission yeast. *Nature* 381(6581):438–441
- Funabiki H, Yamano H, Nagao K, Tanaka H, Yasuda H, Hunt T, Yanagida M (1997) Fission yeast Cut2 required for anaphase has two destruction boxes. *EMBO J* 16(19):5977–5987
- Fuss H, Dubitzky W, Downes CS, Kurth MJ (2005) Mathematical models of cell cycle regulation. *Brief Bioinf* 6(2):163–177
- Gardner TS, Dolnik M, Collins JJ (1998) A theory for controlling cell cycle dynamics using a reversibly binding inhibitor. *Proc Natl Acad Sci USA* 95(24):14190–14195
- Goldbeter A (1991) A minimal cascade model for the mitotic oscillator involving cyclin and Cdc2 kinase. *Proc Natl Acad Sci USA* 88(20):9107–9111
- Gould KL, Moreno S, Owen DJ, Sazer S, Nurse P (1991) Phosphorylation at Thr167 is required for *Schizosaccharomyces pombe* P34cdc2 function. *EMBO J* 10(11):3297–3309
- Harper JW, Burton JL, Solomon MJ (2002) The anaphase-promoting complex: it's not just for mitosis any more. *Genes Dev* 16(17):2179–2206. doi:[10.1101/Gad.1013102](https://doi.org/10.1101/Gad.1013102)
- Hatzimanikatis V, Lee KH, Bailey JE (1999) A mathematical description of regulation of the G1-S transition of the mammalian cell cycle. *Biotechnol Bioeng* 65(6):631–637
- Hayles J, Nurse P (1989) A review of mitosis in the fission yeast *Schizosaccharomyces pombe*. *Exp Cell Res* 184(2):273–286
- Ingolia NT, Murray AW (2004) The ups and downs of modeling the cell cycle. *Curr Biol* 14(18):R771–R777. doi:[10.1016/j.cub.2004.09.018](https://doi.org/10.1016/j.cub.2004.09.018)
- Iwamoto K, Hamada H, Eguchi Y, Okamoto M (2011) Mathematical modeling of cell cycle regulation in response to DNA damage: exploring mechanisms of cell-fate determination. *Biosystems* 103(3):384–391. doi:[10.1016/j.biosystems.2010.11.011](https://doi.org/10.1016/j.biosystems.2010.11.011)
- Kellogg DR (2003) Wee1-dependent mechanisms required for coordination of cell growth and cell division. *J Cell Sci* 116(24):4883–4890
- Kinoshita N, Ohkura H, Yanagida M (1990) Distinct, essential roles of type-1 and type-2a protein phosphatases in the control of the fission yeast-cell division cycle. *Cell* 63(2):405–415
- Kinoshita N, Yamano H, Niwa H, Yoshida T, Yanagida M (1993) Negative regulation of mitosis by the fission yeast protein phosphatase ppa2. *Genes Dev* 7(6):1059–1071
- Kitamura K, Maekawa H, Shimoda C (1998) Fission yeast Ste9, a homolog of Hct1/Cdh1 and fizzy-related, is a novel negative regulator of cell cycle progression during G(1)-phase. *Mol Biol Cell* 9(5):1065–1080
- Kohn KW (1999) Molecular interaction map of the mammalian cell cycle control and DNA repair systems. *Mol Biol Cell* 10(8):2703–2734
- Kominami K, Seth-Smith H, Toda T (1998) Apc10 and Ste9/Srw1, two regulators of the APC-cyclosome, as well as the CDK inhibitor Rum1 are required for G(1) cell-cycle arrest in fission yeast. *EMBO J* 17(18):5388–5399
- Kumada K, Nakamura T, Nagao K, Funabiki H, Nakagawa T, Yanagida M (1998) Cut1 is loaded onto the spindle by binding to Cut2 and promotes anaphase spindle movement upon Cut2 proteolysis. *Curr Biol* 8(11):633–641
- Labib K, Moreno S (1996) rum1: a CDK inhibitor regulating G1 progression in fission yeast. *Trends Cell Biol* 6(2):62–66
- Labib K, Moreno S, Nurse P (1995) Interaction of Cdc2 and Rum1 regulates start and S-phase in fission yeast. *J Cell Sci* 108:3285–3294
- Labib K, Nurse P (1993) Cell-cycle—bring on the phosphatases. *Curr Biol* 3(3):164–166
- Lee MS, Enoch T, Piwnicaworms H (1994) Mik1(+) encodes a tyrosine kinase that phosphorylates P34(Cdc2) on Tyrosine-15. *J Biol Chem* 269(48):30530–30537
- Li SH, Brazhnik P, Sobral B, Tyson JJ (2009) Temporal controls of the asymmetric cell division cycle in *caulobacter crescentus*. *Plos Comput Biol* 5(8). doi:[10.1371/journal.pcbi.1000463](https://doi.org/10.1371/journal.pcbi.1000463)

- Li B, Shao B, Yu CL, Ouyang Q, Wang HL (2010) A mathematical model for cell size control in fission yeast. *J Theor Biol* 264(3):771–781. doi:[10.1016/j.jtbi.2010.03.023](https://doi.org/10.1016/j.jtbi.2010.03.023)
- Lovrics A, Csikasz-Nagy A, Zsely IG, Zador J, Turanyi T, Novak B (2006) Time scale and dimension analysis of a budding yeast cell cycle model. *Bmc Bioinf* 7. doi:[10.1186/1471-2105-7-494](https://doi.org/10.1186/1471-2105-7-494)
- Lundgren K, Walworth N, Booher R, Dembski M, Kirschner M, Beach D (1991) Mik1 and Wee1 cooperate in the inhibitory tyrosine phosphorylation of Cdc2. *Cell* 64(6):1111–1122
- MacNeil SA, Nurse P (1997) Cell cycle control in fission yeast, vol 3. the molecular and cellular biology of the yeast *Saccharomyces*: cell cycle and cell biology. Cold Spring Harbor Laboratory Press, New York
- Marlovits G, Tyson CJ, Novak B, Tyson JJ (1998) Modeling M-phase control in *Xenopus* oocyte extracts: the surveillance mechanism for unreplicated DNA. *Biophys Chem* 72(1–2):169–184
- Mendenhall MD, Hodge AE (1998) Regulation of cdc28 cyclin-dependent protein kinase activity during the cell cycle of the yeast *Saccharomyces cerevisiae*. *Microbiol Mol Biol Rev* 62(4):1191
- Millar JBA, Lenaers G, Russell P (1992) Pyp3-Ptpase acts as a mitotic inducer in fission yeast. *EMBO J* 11(13):4933–4941
- Morgan DO (1995) Principles of Cdk regulation. *Nature* 374(6518):131–134
- Moser BA, Russell P (2000) Cell cycle regulation in *Schizosaccharomyces pombe*. *Curr Opin Microbiol* 3(6):631–636
- Murray AW, Kirschner MW (1989) Cyclin synthesis drives the early embryonic-cell cycle. *Nature* 339(6222):275–280
- Nasmyth K (2001) A prize for proliferation. *Cell* 107(6):689–701
- Norel R, Agur Z (1991) A model for the adjustment of the mitotic clock by cyclin and Mpf levels. *Science* 251(4997):1076–1078
- Novak B, Mitchison JM (1986) Change in the rate of CO₂ production in synchronous cultures of the fission yeast *Schizosaccharomyces pombe*—a periodic cell-cycle event that persists after the DNA-division cycle has been blocked. *J Cell Sci* 86:191–206
- Novak B, Tyson JJ (1993) Modeling the cell-division cycle—M-phase trigger, oscillations, and size control. *J Theor Biol* 165(1):101–134
- Novak B, Tyson JJ (1995) Quantitative-analysis of a molecular-model of mitotic control in fission yeast. *J Theor Biol* 173(3):283–305
- Novak B, Tyson JJ (1997) Modeling the control of DNA replication in fission yeast. *Proc Natl Acad Sci USA* 94(17):9147–9152
- Novak B, Tyson JJ (2008) Design principles of biochemical oscillators. *Nat Rev Mol Cell Biol* 9(12):981–991. doi:[10.1038/Nrm2530](https://doi.org/10.1038/Nrm2530)
- Novak B, Csikasz-Nagy A, Gyorffy B, Chen K, Tyson JJ (1998) Mathematical model of the fission yeast cell cycle with checkpoint controls at the G1/S, G2/M and metaphase/anaphase transitions. *Biophys Chem* 72(1–2):185–200
- Novak B, Toth A, Csikasz-Nagy A, Gyorffy B, Tyson JJ, Nasmyth K (1999) Finishing the cell cycle. *J Theor Biol* 199(2):223–233
- Novak B, Pataki Z, Ciliberto A, Tyson JJ (2001) Mathematical model of the cell division cycle of fission yeast. *Chaos* 11(1):277–286
- Novak B, Kapuy O, Domingo-Sananes MR, Tyson JJ (2010) Regulated protein kinases and phosphatases in cell cycle decisions. *Curr Opin Cell Biol* 22(6):801–808. doi:[10.1016/j.ceb.2010.07.001](https://doi.org/10.1016/j.ceb.2010.07.001)
- Nurse P (1990) Universal control mechanism regulating onset of M-phase. *Nature* 344(6266):503–508
- Nurse P (1991) The Florey lecture, 1990—how is the cell-division cycle regulated. *Philos Trans R Soc Lond B-Biol Sci* 332(1264):271–276
- Nurse P (2000) A long twentieth century of the cell cycle and beyond. *Cell* 100(1):71–78
- Nurse P (2002) Cyclin dependent kinases and cell cycle control (nobel lecture). *ChemBiochem* 3(7):596
- Nurse P (2003) Understanding cells. *Nature* 424(6951):883. doi:[10.1038/424883a](https://doi.org/10.1038/424883a)
- Nurse P, Thuriaux P (1980) Regulatory genes-controlling mitosis in the fission yeast *Schizosaccharomyces pombe*. *Genetics* 96(3):627–637
- Obeyesekere MN, Zimmerman SO, Tecarro ES, Auchmuty G (1999) A model of cell cycle behavior dominated by kinetics of a pathway stimulated by growth factors. *Bull Math Biol* 61(5):917–934
- Oliva A, Rosebrock A, Ferrezuelo F, Pyne S, Chen HY, Skiena S, Futcher B, Leatherwood J (2005) The cell cycle-regulated genes of *Schizosaccharomyces pombe*. *Plos Biol* 3(7):1239–1260. doi:[10.1371/journal.pbio.0030225](https://doi.org/10.1371/journal.pbio.0030225)
- Pallen CJ, Tan YH, Guy GR (1992) Protein phosphatases in cell signalling. *Curr Opin Cell Biol* 4:1000–1007
- Peng X, Karuturi RKM, Miller LD, Lin K, Jia YH, Kondu P, Wang L, Wong LS, Liu ET, Balasubramanian MK, Liu JH (2005) Identification of cell cycle-regulated genes in fission yeast. *Mol Biol Cell* 16(3):1026–1042. doi:[10.1091/mbc.E04-04-0299](https://doi.org/10.1091/mbc.E04-04-0299)
- Qu ZL, MacLellan WR, Weiss JN (2003) Dynamics of the cell cycle: checkpoints, sizers, and timers. *Biophys J* 85(6):3600–3611
- Queralt E, Uhlmann F (2008) Separase cooperates with Zds1 and Zds2 to activate Cdc14 phosphatase in early anaphase. *J Cell Biol* 182(5):873–883. doi:[10.1083/jcb.200801054](https://doi.org/10.1083/jcb.200801054)
- Queralt E, Lehane C, Novak B, Uhlmann F (2006) Downregulation of PP2A(Cdc-95) phosphatase by separase initiates mitotic exit in budding yeast. *Cell* 125(4):719–732. doi:[10.1016/j.cell.2006.03.038](https://doi.org/10.1016/j.cell.2006.03.038)
- Rupes I (2002) Checking cell size in yeast. *Trends Genet* 18(9):479–485
- Rustici G, Bahler J (2001) Study of global gene expression patterns during the fission yeast cell cycle using DNA microarrays. *Yeast* 18:S99
- Rustici G, Mata J, Kivinen K, Lio P, Penkett CJ, Burns G, Hayles J, Brazma A, Nurse P, Bahler J (2004) Periodic gene expression program of the fission yeast cell cycle. *Nat Genet* 36(8):809–817. doi:[10.1038/Ng1377](https://doi.org/10.1038/Ng1377)
- Shimanuki M, Kinoshita N, Ohkura H, Yoshida T, Toda T, Yanagida M (1993) Isolation and characterization of the fission yeast protein phosphatase gene ppe1+ involved in cell-shape control and mitosis. *Mol Biol Cell* 4(3):303–313
- Sible JC, Tyson JJ (2007) Mathematical modeling as a tool for investigating cell cycle control networks. *Methods* 41(2):238–247. doi:[10.1016/j.ymeth.2006.08.003](https://doi.org/10.1016/j.ymeth.2006.08.003)
- Srividhya J, Gopinathan MS (2006) A simple time delay model for eukaryotic cell cycle. *J Theor Biol* 241(3):617–627. doi:[10.1016/j.jtbi.2005.12.020](https://doi.org/10.1016/j.jtbi.2005.12.020)
- Stegmeier F, Amon A (2004) CLOSING MITOSIS: The functions of the Cdc14 phosphatase and its regulation. *Annu Rev Genet* 38:203–232
- Stern B, Nurse P (1996) A quantitative model for the cdc2 control of S phase and mitosis in fission yeast. *Trends Genet* 12(9):345–350
- Sveiczzer A, Novak B, Mitchison JM (1999) Mitotic control in the absence of cdc25 mitotic inducer in fission yeast. *J Cell Sci* 112(7):1085–1092
- Sveiczzer A, Csikasz-Nagy A, Gyorffy B, Tyson JJ, Novak B (2000) Modeling the fission yeast cell cycle: quantized cycle times in wee1(–) cdc25 Delta mutant cells. *Proc Natl Acad Sci USA* 97(14):7865–7870
- Toth A, Queralt E, Uhlmann F, Novak B (2007) Mitotic exit in two dimensions. *J Theor Biol* 248(3):560–573. doi:[10.1016/j.jtbi.2007.06.014](https://doi.org/10.1016/j.jtbi.2007.06.014)
- Trautmann S, McCollum D (2002) Cell cycle: new functions for Cdc14 family phosphatases. *Curr Biol* 12(21):R733–R735
- Trautmann S, McCollum D (2005) Distinct nuclear and cytoplasmic functions of the S-pombe Cdc14-like phosphatase Clp1p/Flp1p

- and a role for nuclear shuttling in its regulation. *Curr Biol* 15(15):1384–1389. doi:[10.1016/j.cub.2005.06.039](https://doi.org/10.1016/j.cub.2005.06.039)
- Trautmann S, Wolfe BA, Jorgensen P, Tyers M, Gould KL, McCollum D (2001) Fission yeast Clp1p phosphatase regulates G2/M transition and coordination of cytokinesis with cell cycle progression. *Curr Biol* 11(12):931–940
- Trautmann S, Rajagopalan S, McCollum D (2004) The *S. pombe* Cdc14-like phosphatase Clp1p regulates chromosome biorientation and interacts with Aurora Kinase. *Dev Cell* 7(5):755–762
- Trinkle-Mulcahy L, Lamond AI (2006) Mitotic phosphatases: no longer silent partners. *Curr Opin Cell Biol* 18(6):623–631. doi:[10.1016/j.ceb.2006.09.001](https://doi.org/10.1016/j.ceb.2006.09.001)
- Tyson JJ (1991) Modeling the cell-division cycle—Cdc2 and cyclin interactions. *Proc Natl Acad Sci USA* 88(16):7328–7332
- Tyson JJ, Novak B (2001) Regulation of the eukaryotic cell cycle: molecular antagonism, hysteresis, and irreversible transitions. *J Theor Biol* 210(2):249–263
- Tyson JJ, Novak B (2008) Temporal organization of the cell cycle. *Curr Biol* 18(17):R759–R768. doi:[10.1016/j.cub.2008.07.001](https://doi.org/10.1016/j.cub.2008.07.001)
- Tyson JJ, Novak B (2010) Functional motifs in biochemical reaction networks. *Ann Rev Phys Chem* 61(61):219–240. doi:[10.1146/annurev.physchem.012809.103457](https://doi.org/10.1146/annurev.physchem.012809.103457)
- Tyson JJ, Novak B (2011) Cell cycle: who turns the crank? *Curr Biol* 21(5):R185–R187. doi:[10.1016/j.cub.2011.01.042](https://doi.org/10.1016/j.cub.2011.01.042)
- Tyson JJ, Novak B, Odell GM, Chen K, Thron CD (1996) Chemical kinetic theory: understanding cell cycle regulation. *Trends Biochem Sci* 21(3):89–96
- Tyson JJ, Chen K, Novak B (2001) Network dynamics and cell physiology. *Natl Rev Mol Cell Biol* 2(12):908–916
- Tyson JJ, Csikasz-Nagy A, Novak B (2002) The dynamics of cell cycle regulation. *Bioessays* 24(12):1095–1109. doi:[10.1002/Bies.10191](https://doi.org/10.1002/Bies.10191)
- Tyson JJ, Chen KC, Novak B (2003) Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell. *Curr Opin Cell Biol* 15(2):221–231. doi:[10.1016/S0955-0674\(03\)00017-6](https://doi.org/10.1016/S0955-0674(03)00017-6)
- Tyson JJ, Albert R, Goldbeter A, Ruoff P, Sible J (2008) Biological switches and clocks. *J R Soc Interface* 5:S1–S8. doi:[10.1098/rsif.2008.0179.focus](https://doi.org/10.1098/rsif.2008.0179.focus)
- Vanoosthuyse V, Hardwick KG (2009) Overcoming inhibition in the spindle checkpoint. *Genes Dev* 23(24):2799–2805. doi:[10.1101/Gad.1882109](https://doi.org/10.1101/Gad.1882109)
- Vasireddy R, Biswas S (2004) Modeling gene regulatory network in fission yeast cell cycle using hybrid Petri nets. *Neural Inf Process* 3316:1310–1315
- Vinod PK, Freire P, Rattani A, Ciliberto A, Uhlmann F, Novak B (2011) Computational modelling of mitotic exit in budding yeast: the role of separase and Cdc14 endocycles. *J R Soc Interface* 8(61):1128–1141. doi:[10.1098/rsif.2010.0649](https://doi.org/10.1098/rsif.2010.0649)
- Wolfe BA, Gould KL (2004) Fission yeast Clp1p phosphatase affects G(2)/M transition and mitotic exit through Cdc25p inactivation. *EMBO J* 23(4):919–929. doi:[10.1038/sj.emboj.7600103](https://doi.org/10.1038/sj.emboj.7600103)
- Wolfe BA, McDonald WH, Yates JR, Gould KL (2006) Phosphoregulation of the Cdc14/Clp1 phosphatase delays late mitotic events in *S-pombe*. *Dev Cell* 11(3):423–430. doi:[10.1016/j.devcel.2006.07.018](https://doi.org/10.1016/j.devcel.2006.07.018)
- Yamada HY, Matsumoto S, Matsumoto T (2000) High dosage expression of a zinc finger protein, Grt1, suppresses a mutant of fission yeast slp1(+), a homolog of CDC20/p55CDC/Fizzy. *J Cell Sci* 113(22):3989–3999
- Yamaguchi S, Okayama H, Nurse P (2000) Fission yeast Fizzy-related protein srw1p is a G(1)-specific promoter of mitotic cyclin B degradation. *EMBO J* 19(15):3968–3977
- Yamashita YM, Nakaseko Y, Kumada K, Nakagawa T, Yanagida M (1999) Fission yeast APC/cyclosome subunits, Cut20/Apc4 and Cut23/Apc8, in regulating metaphase-anaphase progression and cellular stress responses. *Genes Cells* 4(8):445–463
- Yanagida M (1998) Fission yeast cut mutations revisited: control of anaphase. *Trends Cell Biol* 8(4):144–149
- Zachariae W, Nasmyth K (1999) Whose end is destruction: cell division and the anaphase-promoting complex. *Genes Dev* 13(16):2039–2058
- Zarzov P, Decottignies A, Baldacci G, Nurse P (2002) G(1)/S CDK is inhibited to restrain mitotic onset when DNA replication is blocked in fission yeast. *EMBO J* 21(13):3370–3376
- Zwolak J, Adjerid N, Bagci EZ, Tyson JJ, Sible JC (2009) A quantitative model of the effect of unreplicated DNA on cell cycle progression in frog egg extracts. *J Theor Biol* 260(1):110–120. doi:[10.1016/j.jtbi.2009.05.018](https://doi.org/10.1016/j.jtbi.2009.05.018)