PERIODICA POLYTECHNICA SER. CHEM. ENG. VOL. 50, NO. 2, PP. 81–94 (2006)

DEVELOPING A MATHEMATICAL MODEL FOR THE FISSION YEAST CELL CYCLE: SIMULATING MUTANTS OVEREXPRESSING EITHER *CDC25* OR *WEE1*

Quynh Chi LE THI[1], Ákos SVEICZER[1*], and Béla NOVÁK[1,2]

[1]Department of Agricultural Chemical Technology,
[2] Molecular Network Dynamics Research Group of Hungarian Academy of Sciences,
Budapest University of Technology and Economics,
1111 Budapest, Szt. Gellért tér 4, Hungary.
E-mail addresses: quynhchi79@yahoo.com,
asveiczer@mail.bme.hu,
bnovak@mail.bme.hu
* Author for correspondence: Ákos Sveiczer.
Tel: +36 1 4632349.
Fax: +36 1 4632598.

Received: Feb. 14, 2006

Abstract

During the last decade several mathematical models were constructed to describe the fission yeast cell cycle. In these models, fluctuations of MPF activity were responsible for cell cycle transitions, and they successfully explained the behaviour of wild-type fission yeast cells and many cell division cycle mutants as well. However, the mutants involved in these models were mainly loss-of-function mutants (either temperature-sensitive point mutants or gene deletion ones). By contrast, the phenotypes of several gene overproducing (op) mutants have been published during the last twenty years, like those of $cdc25^{op}$ and $wee1^{op}$ cells (in the case of the latter one, even the effects of different overexpression levels are known). Since Wee1 and Cdc25 is a kinase-phosphatase pair, regulating MPF activity and as a consequence, timing mitotic onset in fission yeast, a detailed mathematical model of the fission yeast cell cycle should be able to simulate these overexpression mutants. Within the framework of this paper, a formerly published model was tested for these mutants. In order to describe properly the behaviour of $cdc25^{op}$ and $wee1^{op}$ mutants, some alterations had to be made in the original model, both in the parameter values and in the equations. If these corrections have been involved, the newly developed model also maintained its capability to explain the phenotypes of all those mutants, for which the original model was made. Furthermore, the model predicts the phenotypes of two mutants not yet constructed by geneticists.

Keywords: cell cycle, fission yeast, gene overexpressing mutants, mathematical model, computer simulation.

1. Introduction

The most fundamental phenomenon for life is cell reproduction. Cell cycle is the sequence of events by which a growing cell duplicates all its components and partitions them more-or-less evenly between two daughter cells. The events of the cell cycle are strictly regulated: the initiation of late events is dependent on the

completion of early ones, what is ensured by so-called checkpoint mechanisms. For example, chromosome segregation in eukaryotes is dependent on the completion of DNA synthesis. These processes are controlled by a considerably complicated molecular network called the cell cycle machinery. The most important component in this machinery is *m*aturation *p*romoting *f*actor (MPF), which is a heterodimer composed of a B-type cyclin (called Cdc13 in fission yeast) and a cyclin-dependent kinase (called Cdc2 in fission yeast) [16]. The Cdc2/Cdc13 complex is responsible for phosphorylating several target proteins; its activity is required for firing of replication origins, and also for chromosome condensation and organization of the mitotic spindle. As a consequence, MPF is able to drive fission yeast cells into either S phase (DNA replication) or M phase (mitosis), depending on its level [3, 12].

The activity of MPF fluctuates in cycling cells: it is very low in G1, has an intermediate level in S and G2, and reaches its maximum during M phase [1]. This oscillation is regulated through MPF's interactions with its negative and positive regulators, called enemies and helpers, respectively. The enemies are the Wee1 and Mik1 kinases, the Rum1 inhibitor and *a*naphase *p*romoting *c*omplex (APC), while the helpers are the Cdc25 and Pyp3 phosphatases and some cyclin-dependent kinases, which are dimers of Cdc2 and some other cyclins [16]. From the prospect of this paper, the most important MPF regulators are Wee1 and Cdc25, a kinase-phosphatase pair, which mostly determine the process how a G2 phase cell proceed into M phase. Both Wee1 and Cdc25 are gene-dosage-dependent regulators, and the phenotypes of either loss-of-function or overexpressing mutants of them have long been known [8]-[11].

The biochemical reaction network, which drives the eukaryotic cell cycle, can be studied by computational methods. Since the publication of a seminal paper by John Tyson [18], many mathematical models have been developed to study the cell cycle in different organisms. A couple of years ago, we published such a model for the fission yeast cell cycle [13], which involved the most important cell cycle mutants of the G2/M transition, however, they were mainly loss-of-function ones. The goal of this study is to test this model, whether it is able to describe quantitatively the behaviour of fission yeast cells overexpressing either *cdc25* or *wee1*. We present a slightly modified version of the former model here, which correctly simulates these overexpressing mutants, besides the mutant set for which the original version was made [13].

2. Computational Methods

The molecular network, which controls fission yeast cell cycle, is shown in *Fig. 1*. Using standard techniques of biochemical reaction kinetics we convert this wiring diagram into a set of ordinary differential equations (*Table 1*). The numerical values of all the parameters used for wild-type cells are also given in *Table 1*. For further general details about modelling the fission yeast cell cycle, see [16]; for the special features of the present model, see [13]. *Fig. 1* and *Tables 1* and

2 are all based on [13], and the differences are clearly indicated (see later). To analyse the behaviour of the dynamic system, we have used the differential equation solver WinPP, which is designed for use with Windows and requires the standard C compiler. Information on installing and using this software can be found at the URL site: www.math.pitt.edu/∼bard/classes/wppdoc/.

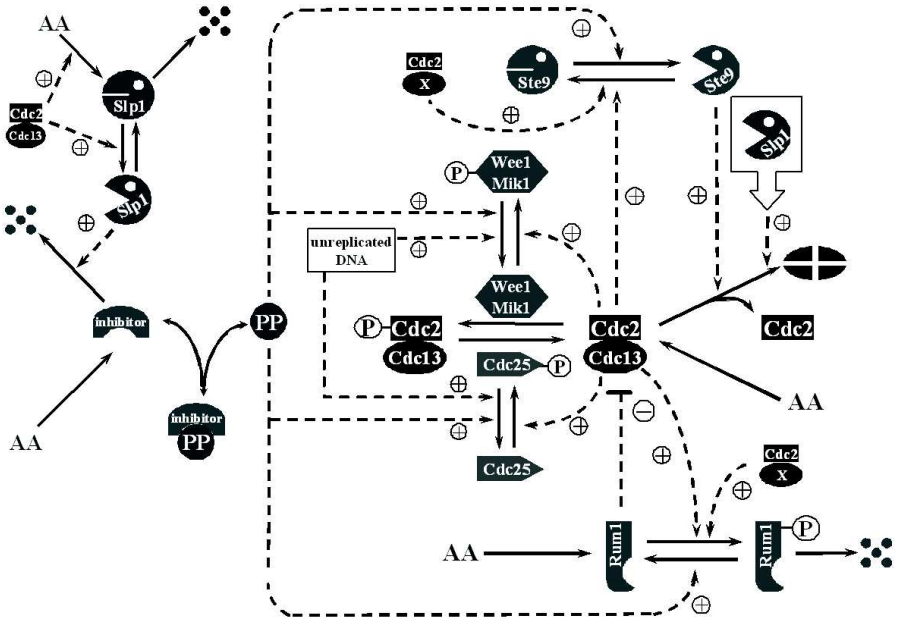

Fig. 1. A molecular mechanism for the regulation of Cdc13-associated kinase activity in fission yeast. All the events of the fission yeast cell cycle can be orchestrated by fluctuations of the activity of a single cyclin-dependent kinase, Cdc2/Cdc13. Cdc13 is synthesized from amino acids (AA) and combines readily with catalytic subunits, Cdc2, which are assumed to be always present in excess. The activity of Cdc2/Cdc13 is modulated by Rum1 inhibition, by Tyr15-phosphorylation (via Wee1 and Mik1, which is reversed by Cdc25 and Pyp3; the last molecule not shown on figure), and by Ste9- and Slp1-dependent cyclin degradation. Compared to a former version, Slp1-dependent Cdc13 degradation is a newly involved biochemical step in the present model, which is emphasized by a black-edged box with an arrowhead. Other cyclins (Puc1, Cig1 and Cig2, which are represented by X in the figure) in complex with Cdc2 can assist these processes; these dimers are clumped together in the model as starter kinases (SK).

3. Results

3.1. The Mathematical Model

As mentioned above, the cell division cycle is controlled by fluctuations in the activity of MPF, which is achieved by its positive and negative regulators. Moreover, MPF affects the activities of its regulators, thereby generating feedback mechanisms in the network of biochemical reactions. In this section, we briefly summarize the most important features of the mathematical model for the fission yeast cell cycle (*Fig. 1* and *Table 1*), on which the present work has been based. For further details about the biochemical roles of the network proteins and other aspects of the model, see [13, 16] and references therein.

During the cell cycle, fission yeast cells continuously synthesize Cdc13 cyclin, which binds to free Cdc2 to form the dimer in the cytoplasm first, but it soon gets into the nucleus. Cdc13's synthesis rate is proportional to cell size (mass), which grows exponentially during the cell cycle. In G1 phase, MPF activity is extremely low, because two of its enemies are very active at this stage. Once Ste9 recognizes Cdc13, which results in its degradation by APC; and secondly, Rum1 inhibits the remaining small amounts of the dimer by stoichiometrically binding to it. At the G1/S transition, both Ste9 and Rum1 become inactivated by Cdc2, complexed partly with Cdc13 and partly with other cyclins (Puc1, Cig1, and Cig2). (For the sake of simplicity, these further dimers are called starter kinases (SK or Cdc2/X) having a constant activity in the model.) During S and G2 phases, Cdc13 is continuously accumulating in the nucleus, since its degradation pathway has been turned off. However, MPF activity reaches only an intermediate level, because its third type enemies, the Wee1 and Mik1 kinases phosphorylate the Cdc2 subunit at its Tyr-15 residue. This form is called preMPF, because although it shows some measurable kinase activity, but it is much weaker compared to the unphosphorylated form (MPF).

In late G2 phase, when the cell has reached a critical mass, and as a consequence, MPF activity has reached a threshold, two positive feedback loops are turned on. MPF inactivates its enemies (Wee1 and Mik1) and activates its helper (Cdc25) by phosphorylating all of them. These feedback loops lead to an abrupt increase in MPF activity, which drives the cell into M phase. However, the high activity MPF peak lasts only for a short period, since a time-delayed negative feedback is turned on by MPF, what causes its own inactivation via Slp1 and a hypothetical phosphatase (PP). (We will turn back to the problem of this negative feedback later.) The decreasing level of MPF allows Rum1 and Ste9 to come back, the cell exits mitosis and the control system falls back into G1 phase with very low MPF activity, and the next cell cycle starts.

3.2. Simulations of Wild-type Fission Yeast Cells and of the Former Mutant Set

A computer simulation of the cell cycle of wild-type fission yeast is shown in *Fig. 2*. These time-courses of the main regulator proteins have been produced by WinPP; using the parameters given in *Table 1*, the software solved the differential equations of *Table 1*. The simulations of consecutive cycles are indistinguishable, since cells from a steady-state population were simulated by a deterministic model. Mass growth during the cell division cycle is proposed to be an exponential function of time, and the relative birth size of simulated wild-type cells is 1, meanwhile that of division size is 2. The profiles of all the proteins shown in *Fig. 2* are consistent with the general characteristics discussed in the previous section as well as with computations by the original model (see *Fig. 2* in [13]). Observe that G1 is a short phase, meanwhile G2 is long (*Fig. 2*), the latter one being the main growth phase in wild-type fission yeast cells. MPF peak during mitosis is very sharp, since the preMPF → MPF transition is a rapid process in late G2 and early M phases, which is very soon followed by MPF inactivation via the negative feedback (MPF → Slp1 → PP → Ste9, leading to Cdc13 degradation). These simulations correctly fit to experimental data [1, 5].

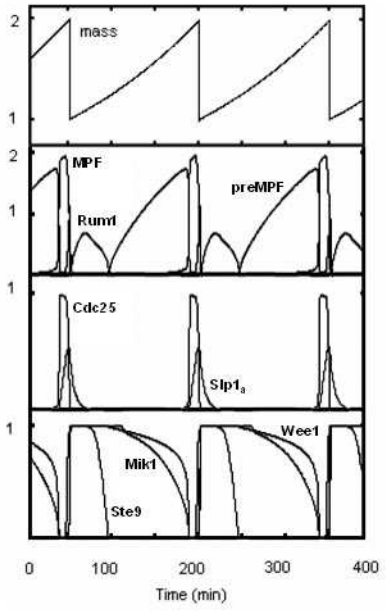

Fig. 2. Numerical simulation of the fission yeast cell cycle (wild type).

Besides wild-type cells, a mathematical model should be able to simulate the phenotypes of cell cycle mutants as well. Simulation of a (single) mutant is

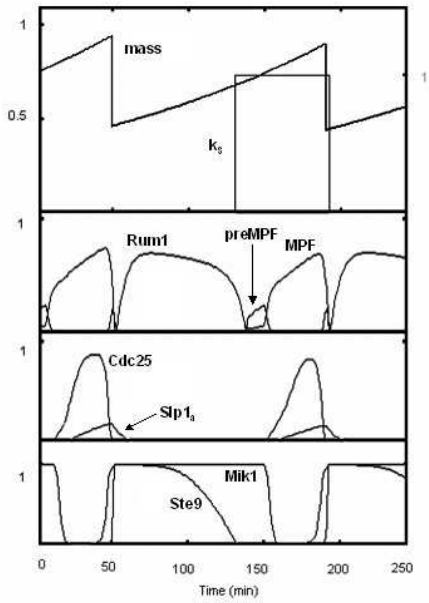

Fig. 3. Numerical simulation of the fission yeast cell cycle ($wee1^{ts}$ $cdc25^{op}$ (adh) mutant). In the upper panel, k_S represents (the abnormally extended S phase.)

achieved by changing the parameter representing the mutation, meanwhile keeping all the other ones the same. The original model [13] was constructed by using a set of 18 single, double or even triple mutants, all of them being loss-of-function ones (bearing either temperature sensitive alleles or gene deletions). In the next two sections we will discuss that the model has required some alterations in order to broaden the mutant set. In this case, an obligatory test for the developed version is to check how far it is still able to simulate the former mutants. In *Table 2*, we give the properties of simulated cell cycles for all the mutants tested, let they be "old" or "novel" ones. Without analysing any of the 18 original strains here, we can establish that the present model correctly describes the behaviour of all of them, similarly to the former version (compare *Table 2* of this paper to *Table 2* of [13]).

3.3. Simulations of Fission Yeast Cells, Overexpressing the cdc25 Gene

Fission yeast cells overexpressing the *cdc25* gene ($cdc25^{op}$ mutants) decrease their size compared to wild-type cells. If the *cdc25* gene was put in five tandem copies into its original place in the genome ($cdc25^{op}(5x)$ mutant), cell size decreased by

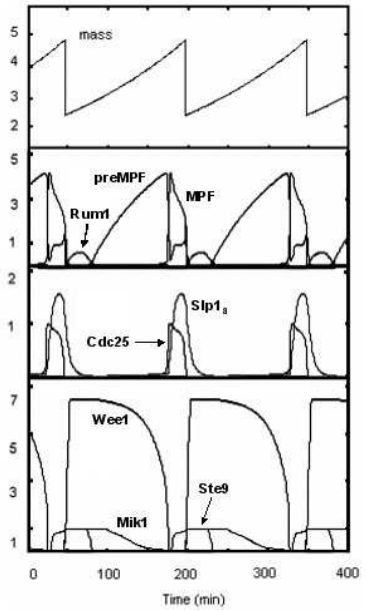

Fig. 4. Numerical simulation of the fission yeast cell cycle (*wee1^{op} (7x)* mutant).

∼25% (semi-wee phenotype); if it was inserted in one copy after a constitutive *adh* promoter (*cdc25^{op}(adh)* mutant), cell size was halved (wee phenotype) [10]. In the previous model, only loss-of-function *cdc25* mutants were studied [13]. In the case of temperature-sensitive alleles, we proposed there that the activities of both the active and inactive forms of Cdc25 protein (V_{25} and V_{25}', respectively) were reduced proportionally, i.e., the ratio of the two rate constants remained the same as in wild-type cells. (Note that Cdc25 is activated by MPF, and inactivated by PP (*Fig. 1*), however, the 'inactive' form also supposed to bear some phosphatase activity.)

Cells overexpressing the *cdc25* gene produce more proteins, which similarly shuttle between the active and inactive forms, depending on the activities of MPF and PP. As a consequence, the simplest way to simulate overproducing mutants is to increase the two forms' rate constants in a proportional way rather than to consider protein levels (inversely to the logic of creating the temperature-sensitive *cdc25* mutant, see above). By multiplying these parameters by 2.25, the simulations exactly described the semi-wee phenotype; while multiplying them by 9 resulted in wee phenotype (*Table 2*). A question should be raised how far these simulations really represent the desired *cdc25^{op}*(5*x*) and *cdc25^{op}(adh)* mutants, because unfortunately there are no experimental data on Cdc25 protein levels in these strains. In the case of *cdc25^{op}*(5*x*), it is very easy to imagine that five tandem gene copies

will not be transcribed and translated to proteins (via mRNAs) so efficiently as a single copy, therefore the actual protein level will be much less (2.25 times) than five times the normal level. In the case of *cdc25^{op}(adh)*, it is even more difficult to evaluate how far a constitutively expressed gene elevates the normal protein level. To our mind, the nine times level of Cdc25 might be realistic.

A further test about the *cdc25^{op}(adh)* mutant is that it is lethal in a *wee1^{-}* background: the *wee1-50 cdc25^{op}(adh)* double mutant executes mitosis and septation before finishing DNA replication and dies (called cut phenotype), at least at 35°C, since *wee1-50* is a temperature-sensitive (ts) allele [10]. In the original model [13], the cut phenotype of other mutants (like *mik1*Δ *wee1-50*, see *Table 2*) was simulated by assuming that if MPF activity exceeded a threshold during S phase, than the speed of DNA replication decreased and the process could not be finished properly before cell division. The model required two small alterations in order to describe correctly the *wee1-50 cdc25^{op}(adh)* double mutant (*Table 2*). Once the critical MPF level at which replication slows down, should have been decreased in order to S and M phases overlap; and secondly, we had to presume that the signal coming from replication forks to activate Mik1 and inactivate Cdc25 (k_{Se}) does not last so long during an abnormally extended S phase (*Fig. 3*). The second correction should have been made, because cut phenotype in this mutant occurs at very small (even smaller than 'normal' wee phenotype) cell size; by contrast, without this change the simulations gave a larger size for 'cut' (not shown). With this model in hand, we can predict the phenotype of the *wee1-50 cdc25^{op}*(5x) double mutant, which, as far as we know, have never been constructed by geneticists. The simulations suggest that in this mutant S and M phases do not overlap (no cut phenotype), however, the cells are probably not viable, because they would divide at an abnormally small cell size (*Table 2*), similarly to the lethal *wee1-50 rum1*Δ double mutant [6, 13, 15]

3.4. Simulations of Fission Yeast Cells, Overexpressing the wee1 Gene

Fission yeast cells with extra copies of the *wee1* gene increase their sizes depending on the number of extra copies [11]. Because G2 phase is extended in these mutants to delay mitotic onset, which phenomenon is more or less proportional to the copy number, *wee1* is considered a gene-dosage dependent inhibitor of mitosis. It is worth emphasizing that in these experiments the extra copies were introduced into the cells on plasmids rather than tandem copies (as in the case of *cdc25*, see previous section). Therefore, it was possible to study the effects of different levels of *wee1* by introducing different numbers of plasmids (*wee1^{op}(3x, 5x, 7x)* see [11]). Wee1 activity is also modulated by MPF: the unphosphorylated form has a large (V_{Wee}), while the phosphorylated form has a small (V_{Wee}') rate constant to convert MPF into preMPF. In the original model, temperature-sensitive loss-of-function *wee1* mutants were simulated by decreasing the active form's rate constant to the value of the inactive form [13]. Unfortunately, this logic cannot be used to simulate the

overproducing mutant; therefore we rather elevated the total level of Wee1 protein in the present model by introducing a new parameter, $Wee1_T$ (*Table 1*). Formerly, Wee1 (similarly to Mik1, Cdc25 or Ste9) was supposed to have a relative total concentration of 1.0 in the cell; here we increase it and check how far simulated cell size fits to the experimental results [11].

When starting to elevate the total Wee1 level, we realized that although $wee1^{op}(3x)$ could be easily simulated by the model, a novel problem arose in higher overexpression. Namely, when the cell entered mitosis with a large cell mass, its MPF peak became so large that the negative feedback mechanisms involved in this model could not win over it, and the cells were blocked in mitosis. This was in sharp contrast to experimental observations [11], meaning that the model required some correction at the level of its wiring diagram rather than in the parameter values. This problem must be connected to the negative feedback loops used in the original model [13]: by the time it was developed, there was no biochemical data regarding the role of Slp1 in mitotic exit, although it was known to be a homologue of the budding yeast Cdc20 [4]. Therefore, only an indirect role of Slp1 was proposed in the model, however, Cdc20 and its further homologues in higher eukaryotes are known to recognize mitotic cyclins for ubiquitination by APC, having a direct role in cyclin degradation at mitotic exit. Soon after the model had been published [13], experiments showed that fission yeast Slp1 probably also took part in Cdc13 degradation [19].

Afterwards, we complemented the differential equations containing MPF turnover by a negative term, whereby Slp1 is directly involved in cyclin degradation (*Table 1*). This effect could have been a weak one only, and even the large cells were able to divide. With this alteration, all the $wee1^{op}$ mutants could have been described, fitting correctly to the experiments (*Fig. 4*, *Table 2*). Observe also that the numerical values of $Wee1_T$ required to simulate these mutants are very close to the *wee1* copy number of the corresponding cells ($Wee1_T$ was 2.4, 4.2 and 6.5 for copy numbers 3, 5 and 7, respectively; *Table 2*). This means that when a gene is inserted into plasmids after its own promoter, then all the copies are expressed efficiently (in contrast to the case, when genes are inserted in tandem repeats after one promoter). Finally, although cells containing more than 7 copies of *wee1* have not been constructed yet, we can raise the question what the phenotype of a $wee1^{op}$*(adh)* mutant could be. Since even the $wee1^{op}(7x)$ mutant is 2.4 times larger than wild type [11], it should probably have a cdc phenotype. However, theoretically this cdc arrest might occur either in late G2 (the cells are unable to enter mitosis) or in mid M phase (they cannot exit mitosis). If $Wee1_T$ was set to 10, the relative division size slightly exceeded 3.0 (*Table 2*), which was considered to be the threshold of cdc phenotype in [13]. Although the duration of mitosis was extended by about a factor of 2, large cell size was obviously caused by delaying mitotic onset rather then exit from it. Moreover, since the direct effect of Slp1 in cyclin degradation is probably even stronger than supposed in the model, we can conclude that the cdc phenotype of a $wee1^{op}$*(adh)* mutant would be probably caused by a G2 block.

4. Discussion

Some years ago, a mathematical model was constructed for the fission yeast cell cycle [13], which correctly described the phenotypes of several loss-of-function mutants of the cell division cycle, mainly of those that had some damage in a gene (or more genes) required for the G2/M transition. This model was the first one, which explained the curious quantized cycles in *wee1^{ts} cdc25*Δ double mutants, observed experimentally by us some years before [14, 15]. We proposed that quantization occurred if the negative feedback mechanism (normally required for mitotic exit) acted prematurely, i.e., cells quitted M phase before properly finishing it. In these abnormal cycles, the negative feedback was itself weak, and the cells returned to G2 rather than G1 (although MPF activity declined, but it stopped at an intermediate level). Slp1 was involved only indirectly in the negative feedback in the original model [13], since this loop was driven mainly by the Tyr-15 kinases and phosphatases of Cdc2 (however, via the hypothetical PP the route finally led to Ste9). The following year after the model had been published, it became clear that although fission yeast really had a phosphatase homologous to budding yeast Cdc14 (called Flp1 or Clp1), but it was not essential for mitotic exit [2, 17]. By that time, it was also known that cyclin degradation was essential for finishing M phase, but it was independent of Ste9 [19]. The most probable hypothesis was that Slp1 was responsible for this Cdc13 degradation, thereby having a direct role in the negative feedback loop. A simple mathematical model was also constructed at that time, which involved this biochemical step and it was able to simulate even quantized cycles, giving an alternative explanation for the mechanism leading to quantization [7].

In the present work we went back to the more detailed model of the fission yeast cell cycle [13] and checked whether it was able to describe mutants overexpressing either the *cdc25* or the *wee1* gene, whose phenotypes had long been known [10, 11]. With some small parameter changes, we could successfully simulate these overexpressors as well as the original mutant set [13], but unfortunately there was one exception. Namely, the simulated *wee1^{op}*$(7x)$ mutant could not divide, as it was rather arrested in mitosis, in sharp contrast to observations [11]. To solve this discrepancy we had to presume exactly the above discussed biochemical reaction (Slp1 directly destroyed Cdc13), and afterwards model fitting to experiments became correct. Although in fission yeast there is no direct experimental evidence to date for the existence of Slp1's direct role against mitotic cyclins, we feel that our theoretical studies strongly suggest such an effect, which is a very important point in eukaryotic cell cycle regulation.

Table 1. A mathematical model of the proposed mechanism (*Fig. 1*) for the fission yeast cell cycle

Differential equations[1]

$\frac{d}{dt}MPF = k_1 \cdot mass + k_{C25} \cdot preMPF + k_{JR} \cdot (CR + CRP) + k'_6 \cdot CR + (k'_6 + k_6) \cdot CRP - k_2 \cdot MPF - k_{Wee} \cdot MPF - k_J \cdot MPF \cdot (Rum1 + Rum1P)$

$\frac{d}{dt}preMPF = k_{Wee} \cdot MPF - k_{C25} \cdot preMPF - k_2 \cdot preMPF$

$\frac{d}{dt}Rum1 = k_5 + (k'_{PP} + k_{PP} \cdot PP) \cdot Rum1P + k_{JR} \cdot CR + k_{2c} \cdot CR - k'_6 \cdot Rum1 - k_P \cdot (MPF_a + \varepsilon_P \cdot SK \cdot mass) \cdot Rum1 - k_J \cdot MPF \cdot Rum1$

$\frac{d}{dt}Rum1P = k_P \cdot (MPF_a + \varepsilon_P \cdot SK \cdot mass) \cdot Rum1 + k_{JR} \cdot CRP + k_{2c} \cdot CRP - (k'_{PP} + k_{PP} \cdot PP) \cdot Rum1P - (k'_6 + k_6) \cdot Rum1P - k_J \cdot MPF \cdot Rum1P$

$\frac{d}{dt}CR = k_J \cdot MPF \cdot Rum1 + (k'_{PP} + k_{PP} \cdot PP) \cdot CRP - k_{JR} \cdot CR - k_{2c} \cdot CR - k'_6 \cdot CR - k_P \cdot (MPF_a + \varepsilon_P \cdot SK \cdot mass) \cdot CR$

$\frac{d}{dt}CRP = k_P \cdot (MPF_a + \varepsilon_P \cdot SK \cdot mass) \cdot CR + k_J \cdot MPF \cdot Rum1P - k_{JR} \cdot CRP - k_{2c} \cdot CRP - (k'_6 + k_6) \cdot CRP - (k'_{PP} + k_{PP} \cdot PP) \cdot CRP$

$\frac{d}{dt}Ste9 = (k'_{Ste9R} + k_{Ste9R} \cdot PP) \cdot \frac{1 - Ste9}{J_{Ste9R} + 1 - Ste9} - k_{Ste9} \cdot (MPF_a + SK \cdot mass) \cdot \frac{Ste9}{J_{Ste9} + Ste9}$

$\frac{d}{dt}Cdc25 = k_{25} \cdot MPF_a \cdot \frac{1 - Cdc25}{J_{25} + 1 - Cdc25} - (k_{Se} + k'_{25R} + k_{25R} \cdot PP) \cdot \frac{Cdc25}{J_{25R} + Cdc25}$

$\frac{d}{dt}Wee1 = (k'_{WR} + k_{WR} \cdot PP) \cdot \frac{Wee1_T - Wee1}{J_{WeeR} + Wee1_T - Wee1} - k_W \cdot MPF_a \cdot \frac{Wee1}{J_{Wee} + Wee1}$

$\frac{d}{dt}Mik1 = (k_{Se} + k'_{MR} + k_{MR} \cdot PP) \cdot \frac{1 - Mik1}{J_{MikR} + 1 - Mik1} - k_M \cdot MPF_a \cdot \frac{Mik1}{J_{Mik} + Mik1}$

$\frac{d}{dt}Slp1_a = (k'_{aa} + k_{aa} \cdot MPF_a) \cdot (Slp1 - Slp1_a) - k_{ai} \cdot Slp1_a - k_{ad} \cdot Slp1_a$

$\frac{d}{dt}Slp1 = k_{as} \cdot MPF_a - k_{ad} \cdot Slp1$

$\frac{d}{dt}Inh = k_3 - k_i \cdot Inh \cdot PP + k_{iR} \cdot PI - k_4 \cdot Inh$

$\frac{d}{dt}PI = k_i \cdot Inh \cdot PP - k_{iR} \cdot PI - k_4 \cdot PI$

$\frac{d}{dt}mass = \mu \cdot mass$

$\frac{d}{dt}R_{DNA} = \frac{K}{1 + Y \cdot MPF_a}$

Rate functions

$k_2 = V'_2 + V_2 \cdot Ste9 + V_2" \cdot Slp1_a$, $k_{2c} = V'_{2c} + V_{2c} \cdot Ste9 + V_{2c}" \cdot Slp1_a$ $\quad k_4 = V'_4 + V_4 \cdot Slp1_a$

$k_{Wee} = V_{Wee} \cdot Wee1 + V'_{Wee} \cdot (Wee1_T - Wee1) + V_{Mik} \cdot Mik1 + V'_{Mik} \cdot (1 - Mik1)$

$k_{C25} = V_{25} \cdot Cdc25 + V'_{25} \cdot (1 - Cdc25) + V_{Pyp} \cdot Pyp3$

Table 1 continued

Auxiliary functions

$MPF_a = MPF + \varepsilon \cdot preMPF \qquad PP = 1 - PI$

Switches

(*i*)When the ratio MPF_a/PP crosses 0.65 from below, S phase is initiated: R_{DNA} is set to 0, and k_S is set to $k_{S,max}$. R_{DNA} represents the fraction of DNA which has been replicated; if it reaches 1, then DNA replication is complete, and k_S is set to $k_{S,min}$. *(ii)* When MPF_a/PP crosses 0.5 from above, the cell divides functionally ($mass \rightarrow mass/2$), although cytokinesis happens much later in fission yeast. *(iii)* In normal cases, MPF activity is low during S phase: $Y = 0$, therefore $dR_{DNA}/dt = K = 0.06$, and replication is fast. However, if *MPF* exceeds the value of the *'cut'* parameter during replication, then Y is set to 100, therefore the rate of replication decreases. During this delayed S phase, if high MPF activity stimulates mitosis and cell division, the cells consequently die (cut phenotype). However, the replication signal (k_{Se}) operating on Cdc25 and Mik1 proteins, is supposed to be turned off earlier (see text).

Rate constants $(min)^{-1}$

$k_5 = 0.1$, $k'_6 = 0.1$, $k_6 = 5$, $k_P = 100$, $k'_{PP} = 1$, $k_{PP} = 100$, $k_J = 400$, $k_{JR} = 1$, $k_1 = 0.02$, $k_{Ste9} = 5$, $k'_{Ste9R} = 0.03$, $k_{Ste9R} = 8$, $k_M = 1$, $k'_{MR} = 0.01$, $k_{MR} = 5$, $k_W = 2$, $k'_{WR} = 0.4$, $k_{WR} = 1$, $k_{25} = 1$, $k'_{25R} = 0.4$, $k_{25R} = 2$, $k_{as} = 0.1$, $k_{ad} = 0.1$, $k'_{aa} = 0.01$, $k_{aa} = 0.1$, $k_{ai} = 0.1$, $k_3 = 0.1$, $k_i = 50$, $k_{iR} = 0.5$, $k_{S,max} = 5$, $k_{S,min} = 0.1$, $V'_2 = 0.02$, $V_2 = 1$,$V_2" = 0.015$, $V'_{2c} = 0.02$, $V_{2c} = 0.5$, $V_{2c}" = 0.0075$, $V_{Wee} = 10$, $V'_{Wee} = 0.08$, $V_{Mik} = 2$, $V'_{Mik} = 0.04$, $V_{25} = 10$, $V'_{25} = 0.05$, $V_{Pyp} = 0.07$, $V'_4 = 0.01$, $V_4 = 1$, $\mu = 0.00462$, $Wee1_T = 1$, $cut = 0.08$

Michaelis and other constants (dimensionless)

$$J_{Ste9} = 0.01,\ J_{Ste9R} = 0.01,\ J_{Mik} = 0.15,\ J_{MikR} = 0.15,\ J_{Wee} = 0.2,$$
$$J_{WeeR} = 0.2,\ J_{25} = 0.05,\ J_{25R} = 0.05,\ SK = 0.018,\ \varepsilon = 0.05,\ \varepsilon_P = 0.025,\ K = 0.06,\ Y = 0,\ Pyp3 = 1$$

[1]Italicised protein names (e.g. *Cdc25*) refer to the concentration of protein, which is a dimensionless number. MPF_a, the total kinase activity of MPF and preMPF; *Rum1P*, concentration of phosphorylated Rum1; *CR*, concentration of Cdc2/Cdc13/Rum1 trimeric complex; *SK*, the activity of starter kinases (Cdc2/X); *CRP*, concentration of Cdc2/Cdc13/Rum1P trimeric complex; $Slp1_a$, the active form of Slp1; *Inh*, concentration of the inhibitor of PP; *PI*, concentration of PP/Inh dimer complex.

Table 2. Simulated cell-cycle properties of mutant fission yeast strains[a]

Strain	Parameter changes[b]	Mass at birth (relative)	G1 (min)	S (min)	G2 (min)	M (min)	Comment
wild-type	–	1.00	38	18	77	17	
wee1^{ts}	$V_{Wee} = 0.08$	0.48	71	19	31	29	
*wee1*Δ	$V_{Wee} = V'_{\mathrm{Wee}} = 0$	0.48	72	19	29	29	
*mik1*Δ	$V_{Mik1} = V'_{\mathrm{Mik}} = 0$	0.97	40	19	72	19	
*mik1*Δ *wee1^{ts}*	$V_{Mik1} = V'_{\mathrm{Mik}} = 0$, $V_{Wee} = 0.08$	No balanced growth, S and M overlap					cut phenotype
*pyp3*Δ	$V_{Pyp} = 0$	1.08	36	19	78	17	
cdc25^{ts} or *cdc25*Δ	$V_{25} = 0.05$, $V'_{25} = 0.00025$ or $V_{25} = V'_{25} = 0$	-	-	-	∞	-	G2-block
*wee1^{ts} cdc25*Δ	$V_{Wee} = 0.08$, $V_{25} = V'_{25} = 0$	1.20/1.03	22/26	20/19	39/103	29/22	quantised cycles
*wee1^{ts} cdc25*Δ *pyp3*Δ	$V_{Wee} = 0.08$, $V_{25} = V'_{25} = 0$, $V_{Pyp} = 0$	-	-	-	∞	-	G2-block
*mik1*Δ *wee1^{ts} cdc25*Δ	$V_{Mik1} = V'_{\mathrm{Mik}} = 0$, $V_{Wee} = 0.08$, $V_{25} = V'_{25} = 0$	No balanced growth, S and M overlap					cut phenotype
wee1^{ts} cdc25^{ts}	$V_{Wee} = 0.08$, $V_{25} = 0.05$, $V'_{25} = 0.00025$	0.80	36	19	68	27	
cdc25^{op} (5x)	$\boldsymbol{V_{25} = 22.5}$, $\boldsymbol{V'_{25} = 0.1125}$	**0.76**	**46**	**19**	**65**	**20**	
wee1^{ts} cdc25^{op} (5x)	$\boldsymbol{V_{Wee} = 0.08}$, $\boldsymbol{V_{25} = 22.5}$, $\boldsymbol{V'_{25} = 0.1125}$	**0.43**	**79**	**20**	**18**	**33**	**probably not viable**[c,d]
cdc25^{op} (adh)	$\boldsymbol{V_{25} = 90}$, $\boldsymbol{V'_{25} = 0.45}$	**0.48**	**70**	**19**	**30**	**31**	
wee1^{ts} cdc25^{op} (adh)	$\boldsymbol{V_{Wee} = 0.08}$, $\boldsymbol{V_{25} = 90}$, $\boldsymbol{V'_{25} = 0.45}$	**No balanced growth, S and M overlap**					**cut phenotype**
wee1^{op} (3x)	$\boldsymbol{Wee1_T = 2.4}$	**1.48**	**32**	**20**	**82**	**16**	
wee1^{op} (5x)	$\boldsymbol{Wee1_T = 4.2}$	**1.93**	**31**	**18**	**81**	**20**	
wee1^{op} (7x)	$\boldsymbol{Wee1_T = 6.5}$	**2.40**	**29**	**19**	**75**	**27**	
wee1^{op} (adh)	$\boldsymbol{Wee1_T = 10}$	**3.02**	**28**	**18**	**70**	**34**	**cdc phenotype**[c]
*wee1*Δ *cdc25*Δ	$V_{Wee} = V'_{\mathrm{Wee}} = 0$, $V_{25} = V'_{25} = 0$	0.76	40	19	64	27	
*rum1*Δ	$k_5 = 0$	0.98	31	19	83	17	
*rum1*Δ *wee1^{ts}*	$k_5 = 0$, $V_{Wee} = 0.08$	0.41	24	19	77	30	not viable[d]
*ste9*Δ	$V_2 = V_{2c} = 0$	0.84	13	20	100	17	
*ste9*Δ *wee1^{ts}*	$V_2 = V_{2c} = 0$, $V_{Wee} = 0.08$	0.30	1	20	97	32	not viable[d]
*ste9*Δ *rum1*Δ	$V_2 = V_{2c} = 0$, $k_5 = 0$	0.83	14	19	100	17	
*ste9*Δ *rum1*Δ *wee1^{ts}*	$V_2 = V_{2c} = 0$, $k_5 = 0$, $V_{Wee} = 0.08$	0.29	0	20	98	32	not viable[d]

[a] The simulation results of the novel (not involved in [13]) mutants are written in bold letters.

[b] Parameter changes relative to the wild-type set (*Table 1*) are indicated.

[c] Model predictions (these mutants have not yet been constructed).

[d] Cells significantly smaller than a *wee1*$^{-}$ mutant are considered too small to be viable.

Acknowledgement

We are grateful to Béla Győrffy for helpful discussions. This research was supported by the Hungarian Scientific Research Fund (OTKA F-034100).

References

[1] CREANOR, J.–MITCHISON, J. M., The Kinetics of H1 Histone Kinase Activation during the Cell Cycle of Wild-type and *wee* Mutants of the Fission Yeast *Schizosaccharomyces pombe*, *J. Cell Sci.*, **107** (1994), pp. 1197–1204.

[2] CUEILLE, N.–SALIMOVA, E.–ESTEBAN, V.–BLANCO, M.–MORENO, S.–BUENO, A.–SIMANIS, V., Flp1, a Fission Yeast Orthologue of the S. Cerevisiae CDC14 Gene, is not Required for Cyclin Degradation or Rum1p Stabilisation at the End of Mitosis, *J. Cell Sci.*, **114** (2001), pp. 2649–2664.

[3] FISHER, D. L.–NURSE, P., A Single Fission Yeast Mitotic Cyclin B $p34^{cdc2}$ Kinase Promotes both S-phase and Mitosis in the Absence of G_1 Cyclins, *EMBO J.*, **15** (1996), pp. 850–860.

[4] MATSUMOTO, T., A Fission Yeast Homolog of CDC20/p55CDC/Fizzy is Required for Recovery from DNA Damage and Genetically Interacts with $p34^{cdc2}$, *Mol. Cell. Biol.*, **17** (1997), pp. 742–750.

[5] MITCHISON, J. M., Cell Cycle Growth and Periodicities, In *Molecular Biology of the Fission Yeast*, A. Nasim, P. Young, B. F. Johnson ed., Academic Press, New York, 1989, pp. 205–242.

[6] MORENO, S.– NURSE, P., Regulation of Progression through the G1 Phase of the Cell Cycle by the $rum1^+$ gene, *Nature*, **367** (1994), pp. 236–242.

[7] NOVAK, B.– PATAKI, Z.– CILIBERTO, A.–TYSON, J. J., Mathematical Model of the Cell Division Cycle of Fission Yeast, *Chaos*, **11** (2001), pp. 277–286.

[8] NURSE, P., Genetic Control of Cell Size at Cell Division in Yeast, *Nature*, **256** (1975), pp. 547–551.

[9] NURSE, P.– THURIAUX, P.–NASMYTH, K., Genetic Control of the Cell Division Cycle in the Fission Yeast *Schizosaccharomyces pombe*, *Mol. Gen. Genet.*, **146** (1976), pp. 167–178.

[10] RUSSELL, P.– NURSE, P., $cdc25^+$ Functions as an Inducer in the Mitotic Control of Fission Yeast, *Cell*, **45** (1986), pp. 145–153.

[11] RUSSELL, P.– NURSE, P., Negative Regulation of Mitosis by $wee1^+$, a Gene Encoding a Protein Kinase Homolog, *Cell*, **49** (1987), pp. 559–567.

[12] STERN, B.– NURSE, P., A Quantitative Model for the cdc2 Control of S Phase and Mitosis in Fission Yeast, *Trends Genet.*, **12** (1996), pp. 345–350.

[13] SVEICZER, A.– CSIKASZ-NAGY, A.– GYŐRFFY, B.– TYSON, J. J.–NOVÁK, B., Modeling the Fission Yeast Cell Cycle: Quantized Cycle Times in $wee1^-$ $cdc25\Delta$ Mutant Cells, *Proc. Natl. Acad. Sci. USA*, **97** (2000), pp. 7865–7870.

[14] SVEICZER, A.– NOVÁK, B.– MITCHISON, J. M., Mitotic Control in the Absence of cdc25 Mitotic Inducer in Fission Yeast, *J. Cell Sci.*, **112** (1999), pp. 1085–1092.

[15] SVEICZER, A.– NOVAK, B.– MITCHISON, J. M., The Size Control of Fission Yeast Revisited, *J. Cell Sci.*, **109** (1996), pp. 2947–2957.

[16] SVEICZER, A.– TYSON, J.– NOVÁK, B., Modelling the Fission Yeast Cell Cycle, *Brief. Funct. Genom. Proteom.*, **2** (2004), pp. 298–307.

[17] TRAUTMANN, S.– WOLFE, B.– JORGENSEN, P.– TYERS, M.– GOULD, K. – MCCOLLUM, D., Fission Yeast Clp1p Phosphatase Regulates G2/M Transition and Coordination of Cytokinesis with Cell Cycle Progression, *Curr. Biol.*, **11** (2001), pp. 931–940.

[18] TYSON, J. J.: Modeling the Cell Division Cycle: cdc2 and cyclin interactions, *Proc. Natl. Acad. Sci. USA*, **88** (1991), pp. 7328–7332.

[19] YAMAGUCHI, S.–OKAYAMA, H.–NURSE, P., Fission Yeast Fizzy-related Protein Srw1p is a G_1-specific Promoter of Mitotic Cyclin B Degradation, *EMBO J.*, **19** (2000), pp. 3968–3977.