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REGULARITIES AND IRREGULARITIES IN THE CELL CYCLE OF THE FISSION YEAST, *SCHIZOSACCHAROMYCES POMBE*

(A REVIEW)

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In an exponentially growing wild-type fission yeast culture a size control mechanism ensures that mitosis is executed only if the cells have reached a critical size. However, there is some scattering both in cell length at birth (BL) and in cycle time (CT). By computational simulations we show here that this scattering cannot be explained solely by asymmetric cell division, therefore we assume that nuclear division is a stochastic, asymmetric process as well. We introduce an appropriate stochastic variable into a mathematical model and prove that this assumption is suitable to describe the CT vs. BL graph in a wild-type fission yeast population. In a double mutant of fission yeast (namely *wee1-50 cdc25Δ*) this CT vs. BL plot is even more curious: cycle time splits into three different values resulting in three clusters in this coordinate system. We show here that it is possible to describe these quantized cycles by choosing the appropriate values of the key parameters of mitotic entry and exit and even more the clustered behavior may be simulated by applying a further stochastic parameter.

Keywords: cell cycle, fission yeast, checkpoints, size control, time-lapse microphotography, deterministic and stochastic modeling, computational simulation, quantized cycles

Introduction

The cell division cycle is the sequence of events whereby a cell duplicates all of its components and divides them between two daughter cells, so that each daughter cell receives the information and machinery necessary to repeat the process. During a

division cycle, the cell must execute the crucial events of chromosome replication and segregation in the correct order (DNA synthesis, followed by mitosis, followed by cytokinesis), and it must ensure that the chromosome replication cycle is synchronized to the mass-doubling cycle. The proper coordination of cell cycle events is enforced at so-called 'checkpoints' [1], where progression through the chromosome cycle is halted until completion of a prior event. The mostly accepted general view is that there are three checkpoints in the eukaryotic cell cycle. The first one is at the G1/S transition or Start (before DNA replication), the second one is at the G2/M transition (before mitosis), and finally the third one is at the metaphase/anaphase transition (during mitosis) [2].

Intensive studies of the physiology of cell division started in the 1950s and yeasts became soon useful model and test organisms in this field. 20 years later genetic methods were introduced for isolating cell cycle mutants of yeasts. By the end of the 1980s biochemists and molecular geneticists had identified most of the central molecular components of the cell-cycle control system, however many new genes and proteins have become known since then [3]. The simple unicellular eukaryote, *Schizosaccharomyces pombe* (fission yeast), has been an attractive model organism in all chapters of cell cycle research, since it is easy to handle by genetic, biochemical, and microscopic methods [4]. The main difference between the industrial baker's yeast (budding yeast, *Saccharomyces cerevisiae*) and fission yeast is the way they grow during their cycle and finally divide [5]. Budding yeast cells have ellipsoidal shape and they generate a bud early in the cycle, and the growing bud finally becomes a (small) daughter cell by peeling off from the (large) mother cell (asymmetric division). In contrast, fission yeast cells have cylindrical shape and they grow exclusively at their tips and finally they make a septum in the middle of the cell producing two nearly identical sister cells (symmetric division).

The advantages of applying fission yeast in cell cycle research are (at least) twofold: (1) symmetric division enables better synchronization; (2) tip growth enables an easy measurement of cell size (since cell diameter is roughly constant, length is proportional to volume and mass). As a further consequence, it is possible to estimate the age (defined by the fraction of the ongoing cell cycle) of a cell simply by measuring its length. The cycle time (CT) of the 'average' cell in an exponentially growing wild-type fission yeast culture is about 150 min under near optimal growth conditions. The average birth length (BL) is 8 μm and length growth ceases when the cell starts mitosis at a size of 14 μm. There is no more growth up to cytokinesis, because cell wall synthesis is restricted to septum formation during the last 20% of the cell cycle [6]. When the septum is partially hydrolyzed, the 14 μm cell divides and the progenies

round off the septum generating two newborn 8 μm cells. The mean cell diameter is ~3.5 μm.

The distribution of the cell cycle phases in wild-type cells is rather unusual (Figure 1). G1 is very short and coincides with the septation period. Cells replicate their DNA at around cell division during a short S phase. The cell spends approximately 70% of its cycle in G2, and finally mitosis (M phase) is the shortest phase. Although all the three checkpoints work in the mitotic cycle of fission yeast, but the rate limiting step is passing through the G2/M checkpoint. The reason is that when cells are growing under more or less optimal nutritional conditions without any perturbation, then the rate limiting step is reaching a critical size before mitosis. The consequence is a long G2 phase. The mechanism which delays a cell cycle event until a critical size has been reached is called a size control mechanism. In general, there are two size controls in the cycle: one at the G1/S checkpoint and another one at the G2/M checkpoint. They ensure size homeostasis in steady-state cultures, i.e. the average birth length of cells cannot differ in successive generations. Both size controls exist in fission yeast, however, in exponentially growing wild-type cells the G1/S size control becomes cryptic [7].

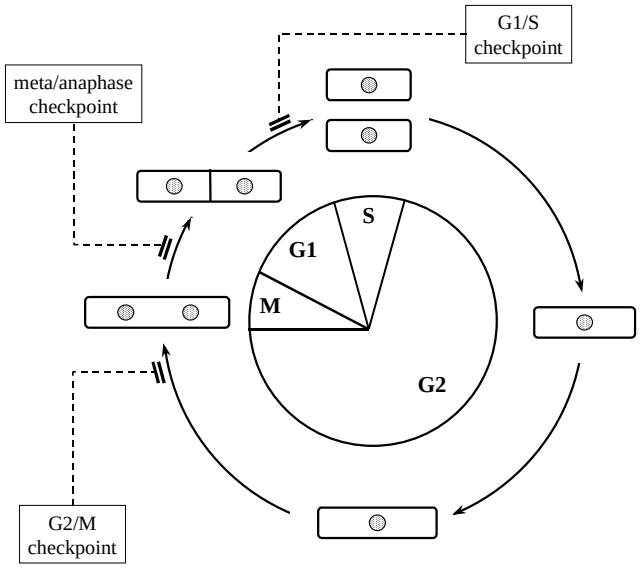

Figure 1. The main characteristics of the cell cycle of the fission yeast, *Schizosaccharomyces pombe*

Cycle time distribution in a wild-type fission yeast culture (experiments)

Time-lapse microphotography is a suitable method to determine length and cycle time data of individual cells (for applications with fission yeast, see [8, 9]). Some years ago we used this method for studying wild-type and many cell cycle mutant populations [10]. The cultures were grown up overnight to mid-exponential phase in minimal medium [11], and then small quantities were placed among a coverslip and a pad of nutrient agar. Photographs were taken by using an automatic timer to take a frame every 5 minutes up to ~8 hours. Measurements were made of birth length (BL), division length (DL) and cycle time (CT) of cells which completed a cycle (in the case of wild-type, we analysed 164 cells).

In accordance with previous papers [8, 9], we established that DL and CT are normally distributed variables in a steady-state culture, the latter one having ~twice the coefficient of variation [10]. This is a sign of size control, since a cell pays more attention on the size of division than that of its time. Plotting CT as a function of BL shows that all the data belong to one cluster (Figure 2). Although there is a relatively large scattering, the negative correlation between the two variables is also obvious: the slope of the regression line ~19.1 min/μm, which is significantly different from 0 ($\alpha = 0.05$). This figure explains how size homeostasis is effectively maintained. Cells with a larger BL shorten their CT, therefore will grow less during their cycle (and vice versa), which ensures a compensation mechanism: if a cell is too large at birth, its progenies will be shorter (and vice versa).

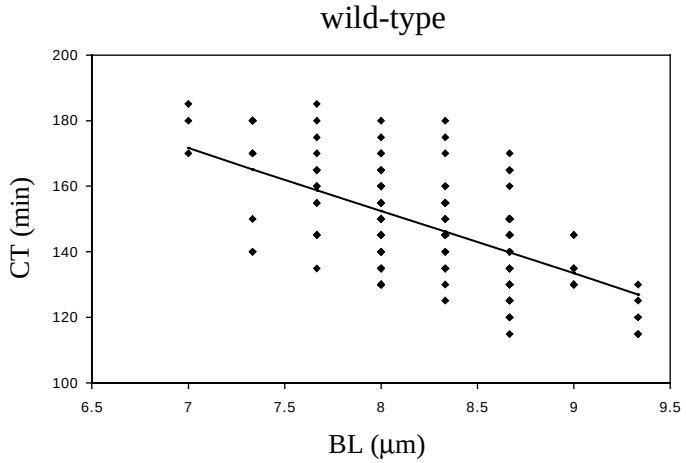

Figure 2. Cycle time (CT) as a function of birth length (BL) in a wild-type fission yeast culture (experiments). Symbols may represent more than one cell; the number of cells belonging to one symbol is much larger in the vicinity of the regression line

Mathematical modeling of the fission yeast cell cycle

We can put a question on why are there differences in BLs and CTs among the cells. If there were a perfect size control before mitosis, all the cells should have equal DLs. Moreover, if cell division were perfectly symmetric, all the newborn cells should have equal BLs. Finally, there were no reasons for variation in CTs. Of course, this simplified view is not supported by experiments (Figure 2), suggesting that there must be an effect(s) causing differences among the cells.

The first idea about this mysterious effect might be that there is some asymmetry in cell division, which is supported by observations, too. However, a large problem arises if we try to explain cycle time and length distributions only by asymmetric cell division, since the cell length difference between the sisters is small [10]. This small asymmetry may cause small standard deviations in BL and CT, and furthermore may not cause any differences in DL [12]. So, we can conclude that although division asymmetry probably has some effect, but it cannot be the main source of length and cycle time scattering in a steady-state population. We should rather imagine that size control is not perfect and should make further efforts in quantitatively describing the events of cell cycle. Mathematical modeling is an appropriate tool to approach such a problem.

Size control in fission yeast is probably sloppy, because a cell cannot measure its length directly and accurately [13]. Inaccurate size control may be a consequence of measuring somehow a parameter whose level is more or less proportional to size (indirect size measurement). An old hypothesis is that cell division is driven by an unknown activator, which is synthesized in the cytoplasm, accumulated in the nucleus, and must reach a critical number in the nucleus [14]. If this activator is unstable and its level is determined by a stochastic birth-and-death process, then different cells accumulate the critical number of activator at different time and size. As a consequence, cycle time and division size also differ [15]. This stochastic ‘black-box’ model explained the cycle time and birth length distributions, moreover predicted the critical number of activator as well as the rate constant of its degradation. Some years ago we made simulations with a simple model of this type and were able to simulate a wild-type fission yeast population with the desired CT vs. BL function [16].

The main disadvantage of the above model is that it neglects the molecular circuitry of cell cycle regulation, which more or less became known during the last two decades. 10 years ago these black-box models were replaced by kinetic ones describing the biochemical reactions in the cell as ordinary differential equations (ODEs) [17]. The first step in developing a model of this type is making a ‘wiring diagram’ consisting of the main components (proteins) and the interactions among them. Next,

the rate of concentration change of every component should be described by an ODE, applying reaction kinetics (law of mass action, enzyme kinetics, etc.). Afterwards, this system of ODEs should be solved numerically by a computer, and the parameters of the ODEs should be set properly. The requirement is that the simulated profile of every component must fit to well-known experimental results. From the mid 90's more and more developed kinetic models of the fission yeast cell cycle were published [18–22] as well as of other organisms (for a very recent review, see [23]).

A simplified version of a wiring diagram of the mitotic cycle of fission yeast is given in Figure 3. The fission yeast cell cycle is driven by fluctuations in the activity of M-phase promoting factor (MPF or Cdc13/Cdc2; shown in the middle of Figure 3), which is a heterodimer of a catalytic subunit (Cdc2 protein kinase) and a regulatory subunit (the B-type cyclin Cdc13). Proper execution of cell cycle events requires that MPF activity oscillates between low (G1 phase), intermediate (S and G2 phases) and high (M phase) levels [24]. These different MPF levels are achieved by antagonistic relationships between MPF and its negative regulators: anaphase promoting complex (APC) and Wee1/Mik1.

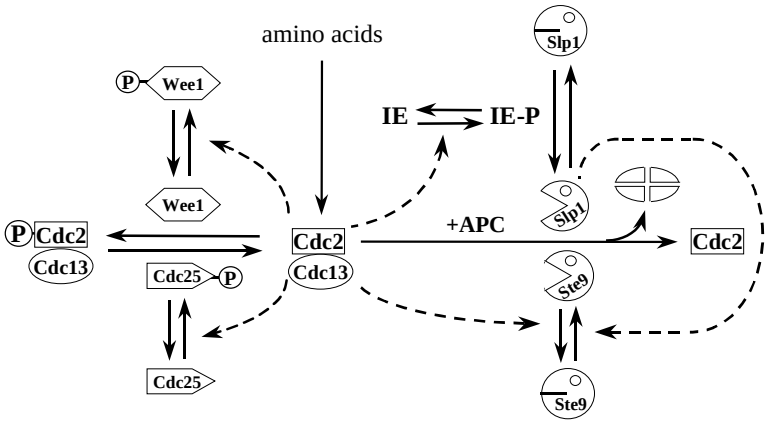

Figure 3. The main part of the wiring diagram of the mathematical model describing the fission yeast cell cycle (regulation of mitotic entry and exit through Cdc13/Cdc2)

In G1 phase, MPF activity is low, because Ste9/APC ubiquitinates the Cdc13 subunit and marks it for degradation [25, 26]. At the G1/S transition, Ste9 is phosphorylated and inactivated by MPF. As a consequence, MPF activity starts to increase, cells pass the Start transition and commence DNA replication (S phase of the cell cycle). However, Cdc13/Cdc2 kinase is not fully activated at this time, because the Tyr-15 residue of Cdc2 is phosphorylated by two tyrosine kinases, Wee1 [27] and

Mik1 [28] (Mik1 is not shown on Figure 3). Since this phosphorylation is inhibitory, MPF activity can reach only intermediate level (S and G2 phase of the cell cycle). In late G2, this inhibitory phosphate group on Tyr-15 is removed by two tyrosine phosphatases, Cdc25 [29] and Pyp3 [30] (Pyp3 is not shown). MPF gets fully activated and this high MPF level drives the cell into mitosis (M phase). MPF activation at the G2/M boundary is accelerated by positive feedback loops, since MPF phosphorylates both Wee1 (this is inhibitory) and Cdc25 (this is activatory), and thereby stimulates its own activation [31].

At the end of mitosis, MPF activity must be eliminated to allow the cell to return to G1 phase of the cycle. Exit from mitosis is induced by a negative feedback loop: MPF indirectly activates Slp1 [32], which inactivates MPF. Slp1 (like Ste9) targets Cdc13 to the APC core, which causes Cdc13 ubiquitination and degradation. Similarly to Ste9, Slp1 is also regulated by MPF dependent phosphorylation. However, in contrast to Ste9, MPF mediated phosphorylation activates Slp1 rather than inactivates it. To create a time delay in the negative feedback, we put an unknown intermediary enzyme (IE) between MPF and Slp1/APC that is reversibly phosphorylated [33]. By reducing MPF activity Slp1 helps the re-activation of Ste9, thereby resetting the cell back to G1.

Although they are important, here we neglect some further components (other cyclins besides Cdc13, the Rum1 stoichiometric inhibitor of MPF, etc.) of cell cycle regulation for simplicity. Cell size is important in cell cycle regulation: since Cdc13 is synthesized in the cytoplasm, forms a dimer with Cdc2 there, and the dimer accumulates in the nucleus, the synthesis of MPF is multiplied by the (relative) cell mass (a larger cell has a larger capacity for protein synthesis). As a consequence, there is a size requirement of reaching a critical MPF activity, which is enough to switch on the positive feedback loops. This model describes the concentration profiles of the main cell cycle regulators in the non-existing ‘average’ wild-type cells [12], but unable to discriminate cells in a culture (deterministic model).

Cycle time distribution in a wild-type fission yeast culture (simulations)

In the previous section we discussed that there were two branches in cell cycle modeling: one of them contained stochastic black-box models, while the other one contained deterministic molecular models. Recently we decided to combine the advantages of the two approaches and make a stochastic, molecular model. We have established that cell division has some asymmetry, which can be considered a stochastic element in the model, but it is not enough to explain the cycle time and

length distributions. Therefore we introduced another stochastic noise into the model, based on the following idea.

Since the effect of Cdc13/Cdc2 complex likely depends on its nuclear concentration (rather than the number of molecules in the nucleus), we introduce a new variable, nuclear volume (NV), and calculate nuclear concentration as (average cytoplasmic concentration)*(cell size)/(nuclear volume). We assume that NV is a normally distributed random variable with mean (μ) = 1.0 (relative value) and a yet unknown standard deviation (σ). After mitosis, a new nucleus is formed, whose volume is chosen from this normal distribution; thereafter, NV is kept constant until the next mitosis. Because there is no experimental data on nuclear volume in fission yeast, we treated σ as an adjustable parameter, chosen to fit CT and DL distributions of the simulated population to experimental data [10]; and a good fit was achieved with $\sigma = 0.07$ [12]. With this stochastic noise, it was possible to simulate a wild-type population having a CT vs. BL graph similar to experiments (Figure 4A). This model has a further interesting characteristic feature. Let us define the nucleocytoplasmic ratio at birth as NCRB = NV/BL. Plotting CT vs. NCRB (Figure 4B) gives a deterministic graph (no scatter), which is linear ($r^2 = 1.0$), implying that NCRB rather than cell size determines inter-division time in this model.

Quantized cell cycles in fission yeast (experiments)

Beside wild-type cells we studied the cycle time distribution in cultures of about 20 cell cycle mutants. In most cases CT, BL and DL parameters were normally distributed and all the cells belonged to one cluster on a CT vs. BL graph [10]. The most notable exception was the *wee1-50 cdc25Δ* double mutant, where CT had a trimodal distribution (the total number of analysed cells was 451). The phenomenon of quantized cycles was even more visible on the CT vs. BL plot (Figure 5A), where the cells formed three clusters (some small ‘wee phenotype’ cells were also observed, but we neglect them here). Analysing these clusters resulted in the conclusion that although there is an apparent weak negative correlation between the variables, size control is missing. Lack of size control is not surprising, since these cells carry the *wee1-50* mutation, which destroys mitotic size control [34]. This mutation causes wee phenotype (~half sized) cells, where a G1/S size control replaces the function of the G2/M size control (the latter one is normally observed in WT cells). However, deletion of the *cdc25* gene in the *wee1-50* background produces larger cells, where the G1/S size control becomes cryptic, but at the same time the G2/M size control is not operating.

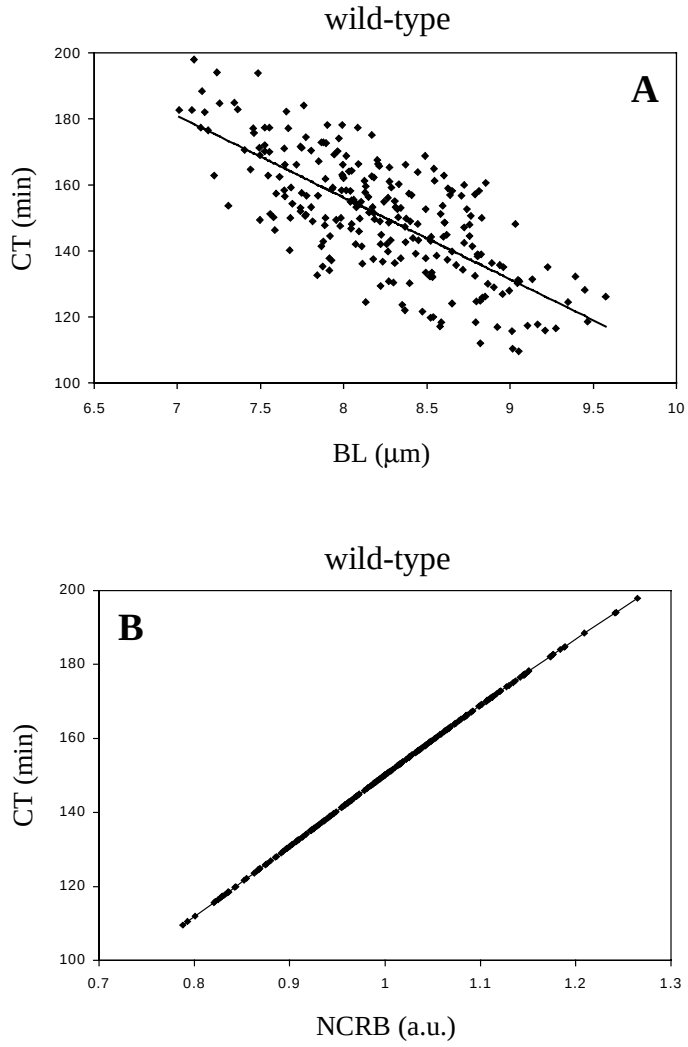

Figure 4. Cycle time (CT) as a function of **(A)** birth length (BL) and of **(B)** nucleocytoplasmic ratio at birth (NCRB) in a wild-type fission yeast culture (simulations)

To get more information about these curious quantized cycles, we determined the length growth patterns of many cells from all the three clusters. The lengths of these cells (~50 in a cluster) were measured in every frame from birth to division. Remember that in wild-type cells length growth ceases at mitotic onset and practically there is no more growth up to cytokinesis (see Introduction). This type of growth

pattern is valid not only in wild-type cells, but, with one exception, in all the cell cycle mutants studied so far by us [10]. The exception is the double mutant *wee1-50 cdc25Δ* [35].

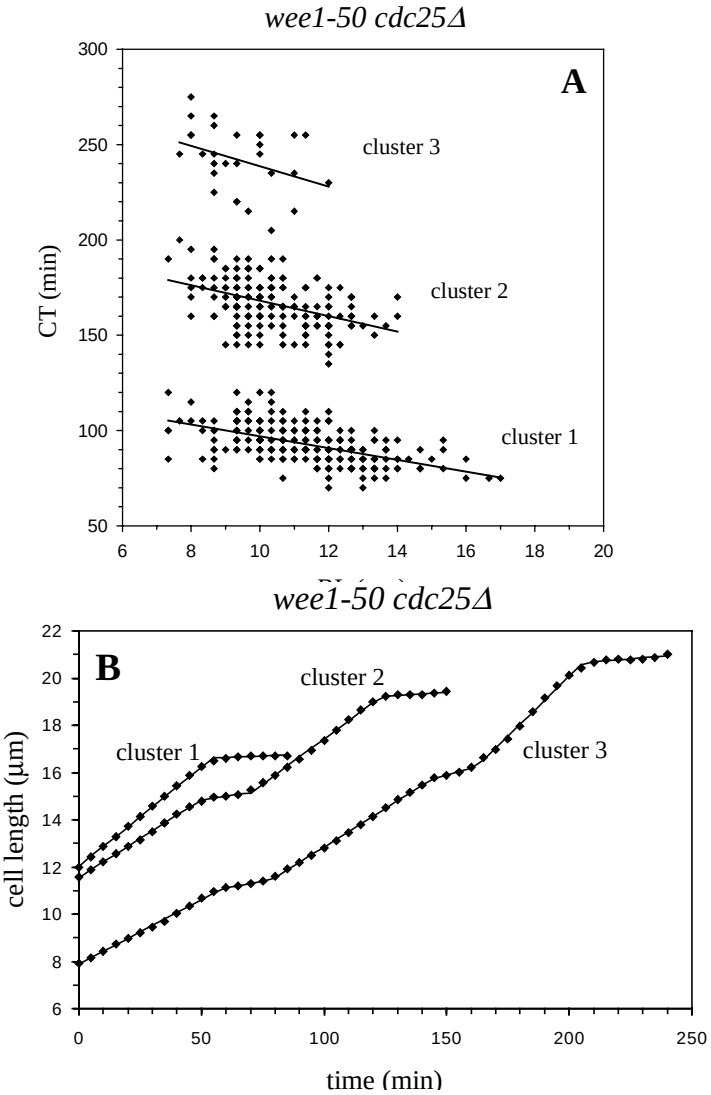

Figure 5. Quantized cell cycles in the *wee1-50 cdc25Δ* double mutant of fission yeast (experiments). **(A)** Cycle time (CT) as a function of birth length (BL); **(B)** length growth patterns of three representative cells

Figure 5B shows typical length growth patterns from all the three clusters of *wee1-50 cdc25Δ*. Cells in cluster 1 have a very short (~90 min) cycle time consisting of a growing period and a plateau at the end of the cycle, like WT. However, cells in cluster 2 with a ~170 min cycle show an interesting pattern having an inner plateau (or at least a slowing down) during the growing period. Note that the duration of this inner plateau is ~half of the duration of the final plateau, and that the slowing down cuts the growing period into two parts more or less symmetrically. In cluster 3, where the mean cycle time is ~240 min, most cells had two inner plateaus, therefore the growing period was divided into five parts: three growing and two non-growing ones. We observed again that the duration of the growing periods were very similar and, at the same time, the non-growing periods lasted up to ~half of the length of the final plateau.

The general conclusion of the results described above was that the cell cycle of the *wee1-50 cdc25Δ* double mutant of fission yeast is often interrupted at an early stage of mitosis from where the cell returns to the beginning of G2 and starts again preparing for mitosis [35]. Instead of executing the total cycle without division more times, these cells pass only the G2 and part of the M phases repeatedly, but neither re-replicate their DNA nor finish nuclear division meanwhile. Since anaphase and the G1/S phases are short in a normal cycle, the cycle times are nearly, but not perfectly doubled. Another problem to be solved is how this population maintains homeostasis in the absence of size control? Observe that the mean cycle time in any cluster is not equal to the mass doubling time of 150 min. The contradiction that this mutant is viable is explained by the observation of random movement of the cells from one cluster to another in successive generations. So, the fact that belonging to a cluster is not an inheritable feature of the cells curiously provides quasi-homeostasis to the whole culture.

Quantized cell cycles in fission yeast (simulations)

The next goal was to develop the former mathematical models in order to be able to describe the quantized cycles observed in *wee1-50 cdc25Δ*. The main idea was the following: in the absence of proteins Cdc25 and Wee1 the positive feedback loops driving the cells from G2 to M phase are very weak. As a consequence, Cdc13/Cdc2 kinase activity cannot increase so abruptly at mitotic onset, therefore early mitotic processes become slow. On the other hand, this kinase activity may prematurely turn on the sound negative feedback loop, which renders the cell back to G2 by decreasing the concentration of MPF, therefore a second (or third, etc.) round of preparation for mitosis starts. When the cell attempts to execute mitosis again, it has a larger size, and therefore has a larger probability of successfully finishing mitosis (remember that accumulation of MPF in the nucleus is thought to be proportional to cell mass). Two

types of these models were recently published, depending on which branch of the negative feedback turns on first: the Mik1 tyrosine kinase [21] or the Slp1/APC dependent Cdc13 proteolysis [22]. In these deterministic models cell size was a bifurcation parameter: cells returned from M phase back to G2 if they were smaller than a critical value, but finished mitosis normally if were larger. This behavior is grossly inconsistent with experiments, although cell mass really has a large impact on the fate of the cell in these quantized cycles [10, 35].

This problem could again be solved by introducing a stochastic element into the model. We observed that in the absence of Cdc25, mitotic onset and exit is severely dependent on the activity of the only remaining phosphatase of Cdc2, Pyp3. Therefore we assumed that the concentration of Pyp3, which was considered constant formerly, should be a stochastic parameter: it is chosen from a random, normally distributed sample at every division and kept constant up to the next division [21]. With this modification in hand, CT is no more determined absolutely by cell size. The standard deviation of Pyp3 concentration is an adjustable parameter; by iterations it is possible to find an apt value for it, with which the model generates a simulated population of *wee1-50 cdc25Δ* showing the phenomenon of quantized cycles. Plotting CT as a function of BL in this populations gives a clustered behavior (Figure 6), comparably to experiments.

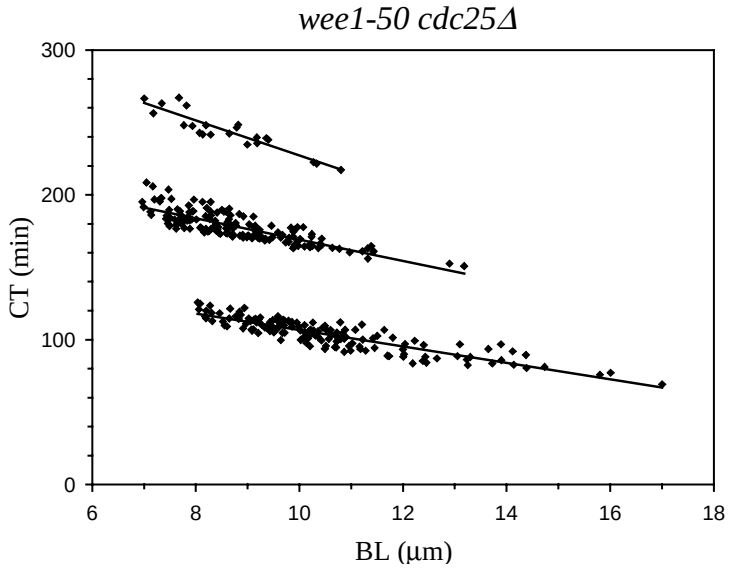

Figure 6. Quantized cell cycles in the *wee1-50 cdc25Δ* double mutant of fission yeast (simulations). Cycle time (CT) as a function of birth length (BL)

Taken together, we argue here that the existence of quantized cycles is a consequence of abnormal MPF oscillations caused by the negative feedback loop mechanisms if the positive feedback loops are compromised. Rendering cells improperly from M to G2 phase depends on two parameters in our model: cell size and the stochastic Pyp3 concentration. This model gives a possible, but experimentally yet not tested explanation for the fact that the *wee1-50 cdc25Δ* population of fission yeast splits into clusters on a CT vs. BL graph.

Discussion

In this paper we summarized our ideas on how cycle time is regulated in fission yeast. All these hypotheses are based on our former experimental or theoretical work, which we made by time-lapse microphotography or mathematical modeling, respectively. Experiments suggested that although birth size of a fission yeast cell has a great impact on its cycle time, there is no pure deterministic relationship between the two variables, since there are stochastic effects involved, too. One level of stochasticity appears even in steady-state cultures, like wild-type cells, when the cells form a cluster in the CT vs. BL plot. A second level of stochasticity was observed exclusively in the double mutant *wee1-50 cdc25Δ*, where cycle times were quantized and the cells formed (at least) 3 different clusters in a similar plot.

When dealing with cycle time distributions in wild-type cells, we first declared that differences among the cells cannot be explained simply by asymmetric cytoplasmic division. Afterwards, we presumed that nuclear division is asymmetric, too, i.e., there is some scattering in nuclear volume. We have chosen nuclear volume as a stochastic parameter, because we believe it is not the quantity of activator (Cdc13/Cdc2) in the nucleus but rather its nuclear concentration that promotes the G2/M transition. In this model, cycle time is perfectly determined by the nucleocytoplasmic ratio at birth, NCRB = NV/BL. This perfect correlation stems from some assumptions in the model (NV is constant between divisions, cell size is an exponential function of time), which are probably not perfectly satisfied, but we propose that the uneven distributions of birth sizes and nuclear volumes of sister cells are the major contributors to cell cycle variability. The idea that cell cycle is under the control of nucleocytoplasmic ratio is a very old one, which has been proved experimentally in many different organisms [36–40].

The second level of stochasticity appears very rarely, since there are only very few cases of observed quantized cycles reported up to now [10, 41]. In fission yeast, the *wee1-50 cdc25Δ* double mutant seems to be the only example on this phenomenon. In this case, we have shown that a deterministic kinetic model might carry the

possibility of an improper resetting from M to G2 phase, when the positive feedback mechanisms needed to mitotic onset become weak, but the negative feedbacks needed to mitotic exit operate normally. By introducing an appropriate stochastic element into this model, it was possible to describe quantitatively the clustered behavior, which is apparent when plotting cycle time as a function of birth length.

Our final conclusion is that random scattering of some critical parameters (which therefore become variables) of the model might cause the experimentally observed distributions of cycle time. These variables are considered to be normally distributed, whose mean values are determined by the behavior of the non-existing average cell, meanwhile the standard deviations are adjustable parameters needed to be set to give the best fit to experiments.

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