

## Mitotic control in the absence of *cdc25* mitotic inducer in fission yeast

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### SUMMARY

Fission yeast cells tolerate the total absence of the *cdc25* mitotic inducer in two cases, either in *cdc2-3w* or in *wee1* genetic backgrounds. In the *cdc2-3w cdc25Δ* double mutant, the rate-limiting step leading to mitosis is reaching a critical size. However, the size control of this mutant operates in late G<sub>2</sub>, which is different from wild-type (WT) cells. This fact suggests that in WT the rate-limiting molecular process during the G<sub>2</sub> timer is the Tyr15 dephosphorylation of *cdc2*, for which the *cdc25* phosphatase (together with its back-up, *pyp3*) is dependent. In the *wee1-50 cdc25Δ* mutant, the population splits into different clusters, all lacking mitotic size control. This strain maintains size homeostasis by a novel method, which is random movement of the cells from one cluster to

another in the successive generations. These cells should normally have a 'minimal cycle', a 'timer' with short G<sub>1</sub> and G<sub>2</sub> phases. However, very often the cells abort mitosis, possibly at an early event and return back to early G<sub>2</sub>, thus lengthening their cycles. The inability of these cells to start anaphase might be caused by the absence of the main mitotic regulators (*wee1* and *cdc25*) and the improper regulation of their back-up copies (*mik1* and *pyp3*, respectively).

Key words: Cell cycle, Cell growth, Cell length, Fission yeast, Homeostasis, Mitotic control, Mitotic inducer (*cdc25/pyp3*), Rate change point, *Schizosaccharomyces pombe*, Size control, Time-lapse film

### INTRODUCTION

The co-ordination of growth and division is an important part of the cell cycle controls, though it has been somewhat neglected in recent years. Such a control is needed to maintain homeostasis of cell size (Fantes and Nurse, 1981).

In a classic paper, Fantes (1977) analysed the results from time-lapse films of individual cells of the wild-type (WT) fission yeast *Schizosaccharomyces pombe*. He showed that there was a homeostatic mechanism which maintained a constant average cell size in growing cultures. This mechanism worked through a 'size control': it was assumed that cells reached a critical threshold size which then allowed mitosis to proceed and it was also believed that this critical size was reached just before mitosis (Fantes and Nurse, 1981). 'Size' was measured as cell length which is proportional to volume in this yeast. However, in a recent paper (Sveiczzer et al., 1996) we established that this 20-year-old description of mitotic size control needed re-examination, and we concluded that this control acts earlier than previously believed, at mid G<sub>2</sub>. The second half of G<sub>2</sub> is a 'timer' during which the cell prepares for mitosis, after the earlier control point when the cell has decided to make these molecular preparations. We use the old phrase 'sizer' for a period when a parameter, such as length is dependent on cell size and 'timer' when it is independent of size.

20 years ago, Nurse and Thuriaux (1977) found a critical

size requirement for DNA replication in the small *wee1* mutant. A little later, Fantes and Nurse (1978) showed that the mitotic size control was absent in *wee1* cells, so identifying *wee1* as a genetic element of this control. However, we gave evidence that *cdc25* is not a genetic element of it (Sveiczzer et al., 1996). We supposed that the *cdc25* mitotic inducer might rather be responsible for one of the main molecular preparations, since it is known to act as a phosphotyrosine phosphatase on Tyr15 of MPF (Millar et al., 1991), therefore activating it.

In this paper, we discuss the situation of how mitotic control works in the absence of *cdc25* (*cdc25Δ* mutants). Fission yeast can survive this obviously lethal condition in two circumstances, i.e. in the absence of mitotic inhibitor *wee1* (Russell and Nurse, 1986) or in the presence of the dominant positive hyperactive allele *cdc2-3w* (Russell and Nurse, 1987). The mechanism of survival is different in the two cases, and by time-lapse analyses of the suitable (double or even triple) cell cycle mutants we give some new information on the physiology of cells lacking *cdc25*.

### MATERIALS AND METHODS

#### Strains, growth and film techniques

The wild-type (WT) strain 972h<sup>-</sup> of *Schizosaccharomyces pombe* was originally obtained from Professor Urs Leupold, Bern. The mutants

used are the derivatives of the original strain (listed in Tables 1 and 2), and were kindly provided by Drs Paul Nurse, Peter Fantes and Jonathan Millar.

The cultures were grown up overnight to  $\sim 2 \times 10^6$  cells/ml at 35°C as described by Sveiczer et al. (1996).

Time-lapse films were made of cells growing between a coverslip and a pad of nutrient agar. The same photomicroscope and techniques were used as described by Sveiczer et al. (1996).

### Analysis

The negative films were projected onto a screen and measurements made of birth length (BL), division length (DL) and cycle time (CT) of cells which completed a cycle. In all cases normality of these variables was tested. These distributions were all normal, except the trimodal cycle time distribution in *wee1-50 cdc25Δ*, as described by Sveiczer et al. (1996). However, the defined subpopulations or clusters of this mutant (see later) showed normal CT distributions. The length extension was calculated as  $Ext = DL - BL$ .

In presenting the plots, the individual cells were grouped but the regression lines were made from the data before grouping. The significance of the slopes of these lines from zero was determined by a *t*-test with  $\alpha = 0.05$ .

The lengths of some cells ( $\sim 50$ -60 in a film or in a cluster of *wee1-50 cdc25Δ*) were measured in every frame from birth to division to get growth patterns. These patterns were smoothed by using the 'rsmooth' command in Minitab. In these smoothed patterns, the times and the cell lengths at the rate change points (RCP), the onset of the constant length period (CL), and the onset and the end of occasional slowing down periods were determined by eye. Regressions were made on the smoothed linear segments before and after an RCP (or inner plateau) and the extent of rate change calculated.

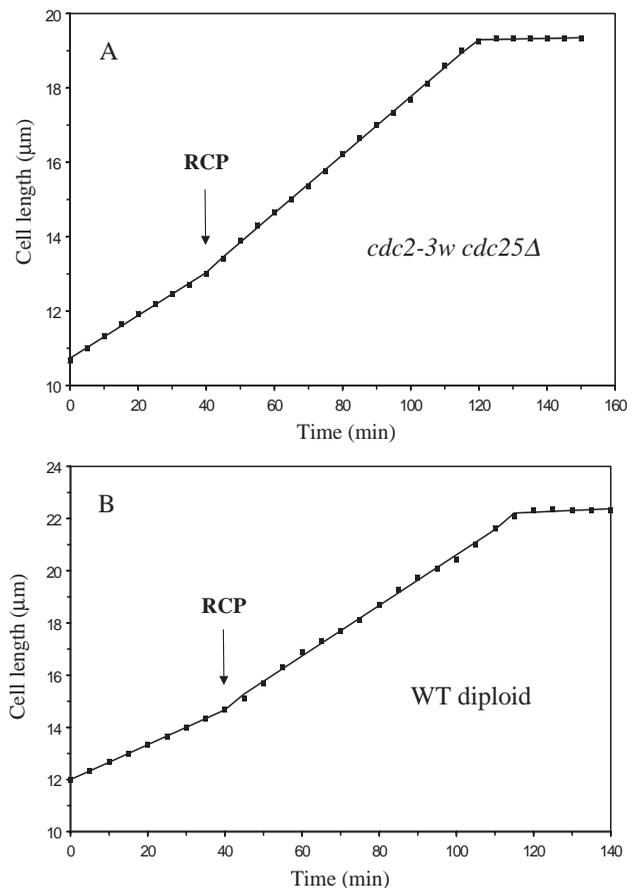
## RESULTS

### Mitotic size control acts after the rate change point in *cdc2-3w cdc25Δ* cells

It has been known for many years that the cylindrical cells of wild-type *S. pombe* grow exclusively at their tips and, to a first approximation, at a constant diameter (Mitchison, 1957; May, 1962; Johnson, 1965). There is general agreement, too, that the cell cycle consists of a growing and a non-growing period, the latter one is called the constant length stage (denoted here by CL), which lasts from mitosis to cell division. Length growth during the growing period is linear with two segments which are separated by a rate change point (RCP) where the rate of length extension increases by  $\sim 30\%$  (Mitchison and Nurse, 1985). Our recent analyses confirmed this idea and extended it to many cell cycle mutants (Sveiczer et al., 1996; see also Mitchison et al., 1998).

Examining the situation of the *cdc2-3w cdc25Δ* double mutant of fission yeast, we first established that these cells showed a typical growth pattern having a 'bilinear' growing stage with an RCP and a CL phase at the end of the cell cycle (Fig. 1A). By using this RCP as a marker, we could divide the cell cycle into two parts, a pre-RCP and a post-RCP one, as described by Sveiczer et al. (1996). The duration of the pre-RCP period is denoted by T1, and the length extension during it is denoted by Ext1, the similar parameters after the RCP are denoted by T2 and Ext2, respectively. Obviously,  $T1 + T2 = CT$ , and  $Ext1 + Ext2 = Ext$ .

According to our previous results (Sveiczer et al., 1996) this double mutant showed a strong mitotic size control. The question here is whether the mitotic size control acts in the



**Fig. 1.** Growth in length of two representative fission yeast cells: (A) *cdc2-3w cdc25Δ* double mutant, and (B) wild-type diploid. RCP, rate change point.

absence of *cdc25* in a similar way to WT. However, Fig. 2A shows that it must be a different way. The regression line of total length extension vs BL graph has a slope not significantly different from  $-1.0$  (see also Table 1), proving again the existence of a strong size control. But, Ext1 is independent of BL and Ext2 is strongly negatively dependent on BL (Fig. 2A); it is just the opposite of WT (see Fig. 3A of Sveiczer et al., 1996). We conclude that the mitotic size control operates in the post-RCP period in this mutant. We have also found that the negative dependence of CT on BL is caused by the fact that the larger cells shorten their post-RCP stage, whereas T1 is a constant 'timer' (Fig. 2B; Table 1). This is consistent with the former figure, and clearly shows that this mutant behaves differently from WT cells (see also Fig. 3B of Sveiczer et al., 1996): there is a timer at the beginning of G<sub>2</sub>, and the sizer is pushed forward well into the second part of G<sub>2</sub>, after the RCP. The reason for these changes might be the absence of *cdc25* (see Discussion).

### Further evidence on the late G<sub>2</sub> control in the *cdc2-3w cdc25Δ* genetic background

To date, the *cdc2-3w* mutant of *S. pombe* is the only known situation where a cell is able to survive the lack of not only the *cdc25* mitotic inducer, but also its back-up phosphatase, *pyp3* (Millar et al., 1992). So, the triple mutant *cdc2-3w cdc25Δ pyp3Δ* is viable, and these cells are even larger than the double

**Table 1. Analysis of rate change points (RCPs)**

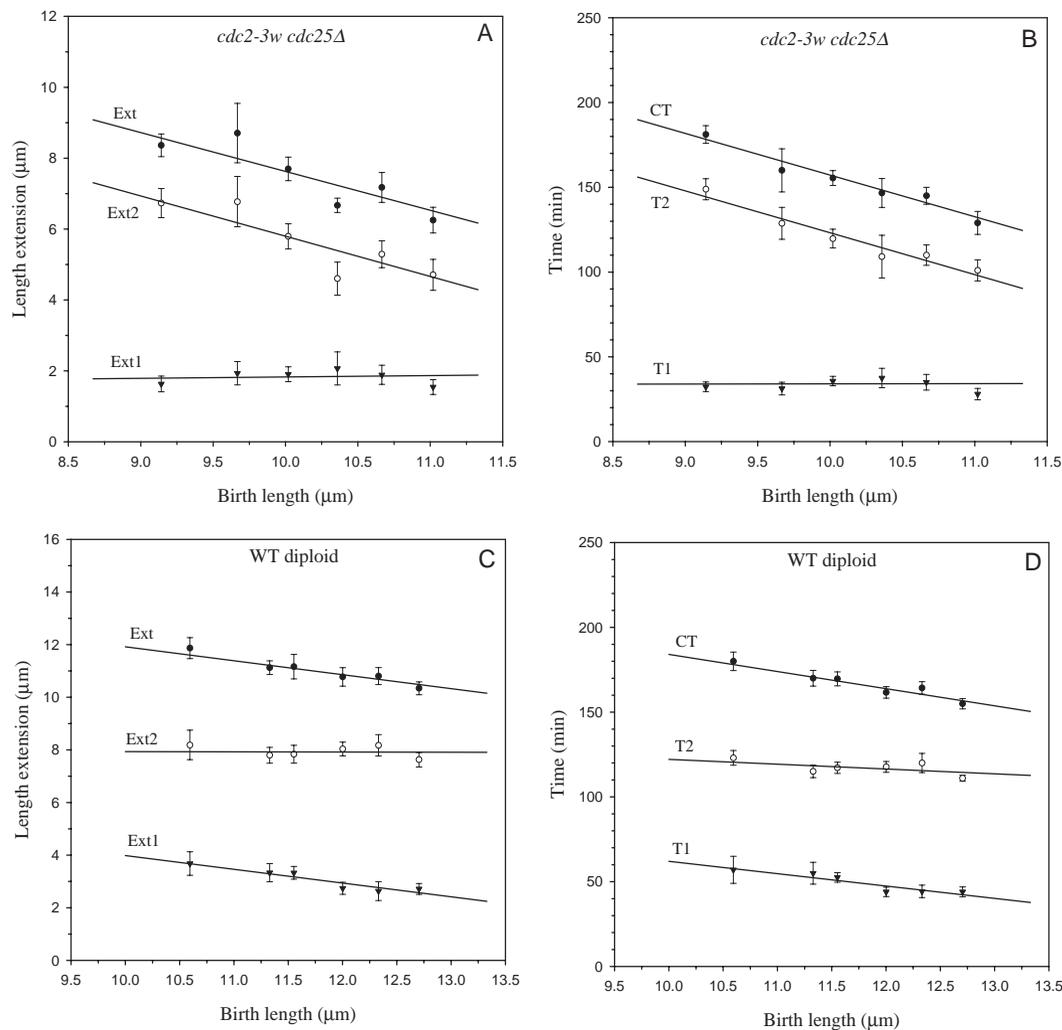
1. Strain (number of cells analysed)	2. Position of RCP (fraction of cycle)	3. Size at RCP ( $\mu\text{m}$ )	4. Increase of rate at RCP (%)	5. CL period (fraction of cycle)	6. Slope Ext1/BL ( $\mu\text{m}/\mu\text{m}$ )	7. Slope Ext2/BL ( $\mu\text{m}/\mu\text{m}$ )	8. Slope T1/BL (min/ $\mu\text{m}$ )	9. Slope T2/BL (min/ $\mu\text{m}$ )
1. <i>cdc2-3w cdc25Δ</i> (58)	0.22	11.9	23	0.20	0.04(NS)	-1.14(S)	0.1(NS)	-24.7(S)
2. <i>cdc2-3w cdc25Δ pyp3Δ</i> (48)	0.28	14.5	26	0.17	-0.11(NS)	-0.93(S)	-0.6(NS)	-14.9(S)
3. WT diploid (57)	0.29	14.9	55	0.18	-0.52(S)	-0.01(NS)	-7.3(S)	-2.8(NS)

Steady-state cultures at 35°C. For method of analysing RCP, see text. Columns 6-9, slopes before and after RCP (for the meaning of the parameters, see text). Superscripts give the significance (S) or not (NS) of the slope deviating from zero.

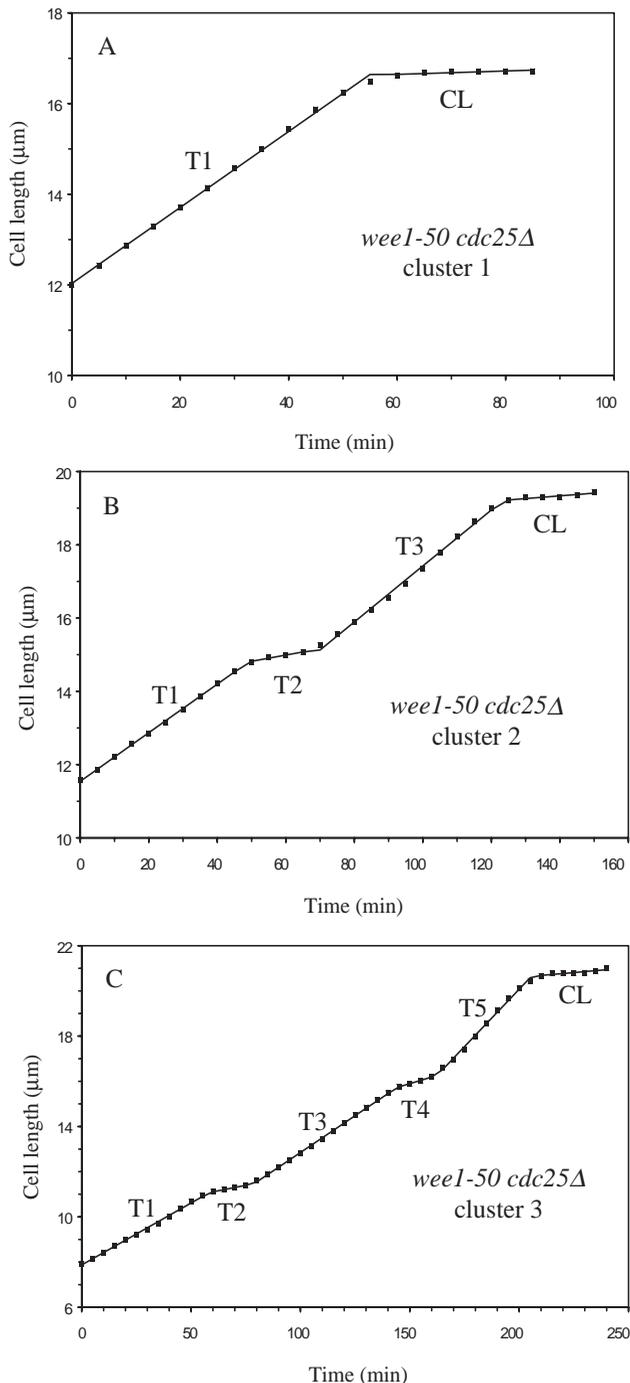
mutant having *pyp3* (Millar et al., 1992), but many cells are sick or die during the time the culture is grown up. On the other hand, size homeostasis is maintained in successive generations by a strong mitotic size control mechanism (Sveiczer et al., 1996). These previous results suggest that the *cdc2-3w* allele might encode a protein having kinase activity even in its Tyr15 phosphorylated form, but, in some cells this activity is probably not enough to execute mitosis.

Length growth during the cell cycle of this triple mutant is very similar to those of the previously discussed double mutant (data not shown). The similarity is also true for the mechanism of the mitotic size control which acts after the rate change point again (Table 1).

Unfortunately, we cannot determine the exact position of this 'late' mitotic size control of these double and triple mutants, but we can make some approximations. The first 0.22-0.28 part of the cycle (the pre-RCP period, Table 1) is a timer, just like the last 0.18-0.20 part (the CL stage, Table 1). So, the middle part between the two timers might be the sizer, or rather it might be made up of a sizer and a timer. There is a classical observation that the sizer period during the average cell of WT fission yeast lasts about 1/3 of the cycle (Miyata et al., 1978b), which is consistent with our recent results (Sveiczer et al., 1996). Since the length variability in these mutants is larger than those of WT, as can be calculated from our data given by Sveiczer et al. (1996), it seems to be a logical assumption that



**Fig. 2.** (A) Extension/birth length in *cdc2-3w cdc25Δ* over the whole cycle and in the periods before and after RCP. (B) A similar plot for time/birth length in *cdc2-3w cdc25Δ*. (C and D) Similar plots for wild-type diploid. Mean values of groups + bar showing s.e.m.



**Fig. 3.** Growth in length of three representative cells of the fission yeast *wee1-50 cdc25Δ* mutant: (A) cluster 1 (B) cluster 2, and (C) cluster 3. For the meaning of the clusters and the T1, ..., T5 parameters, see text.

the sizer part might be extended, too. If the sizer starts soon after the RCP and lasts about 0.33 relative cycle time, then the position of size control in the average cell would be about 0.55–0.61. This is an under-estimate, since if either the sizer is longer or it begins later, this position would move even later in the cycle. As a conclusion, we suggest that the real position of mitotic size control in these mutants is somewhere between 0.60 and 0.75, much later than in the case of WT (~0.34).

Although our results described above indicate that mitotic size control in the absence of *cdc25* (and *pyp3*) operates differently from in WT, one can worry about the larger size of these mutants. Earlier we also analysed large *cdc2* cells after induction synchrony (Svecizer et al., 1996), but they were examined at different temperatures and not in steady-state conditions. Therefore we decided to analyse the situation in WT diploid cells having an intact (WT) genome and a cell size close to the triple mutant. This population maintains homeostasis by a mitotic size control (Svecizer et al., 1996).

The diploid WT cells have the usual (WT like) growth pattern (Fig. 1B). Their mitotic size control is weaker, but acts before the RCP (Fig. 2C; Table 1), and the period after the RCP up to cell division is a timer (Fig. 2D; Table 1). So, the size control mechanism in diploid WT cells is similar to the haploid WT cells, and drastically different from our *cdc25Δ* mutants. It means that this difference has a direct genetic reason (deleted *cdc25*) and it is not a consequence of the larger size.

### Mitotic control in the *wee1-50 cdc25Δ* double mutant

We discussed above the mitotic size control in the absence of *cdc25* which could only have been studied in the *cdc2-3w* background. The other possibility when a cell is able to tolerate the total lack of *cdc25* activity is the *wee1* mutant (Russell and Nurse, 1986). However, it is noteworthy that the viable *wee1-50 cdc25Δ* double mutant becomes lethal if *pyp3* is also deleted (Millar et al., 1992). The next interesting observation was that the population of the *wee1-50 cdc25Δ* double mutant at 35°C consists of cells having an extremely wide length distribution, from *wee* phenotype cells up to even *cdc* phenotype ones (Enoch et al., 1991). Finally, we discovered that cycle time is quantised in this population, and on the CT vs BL graph the cells form (at least four) separate clusters (Svecizer et al., 1996). This strange phenomenon might be connected to the fact that because of the much reduced *wee1* activity mitotic size control does not operate. Below, we give an extended study on this novel behaviour of this double mutant of fission yeast.

#### (1) Lack of size homeostasis in the clusters of the *wee1-50 cdc25Δ* mutant

Based on their CT vs BL graph published by Svecizer et al. (1996) we grouped the cells of this double mutant into four clusters and analysed them separately (Table 2). The CT distributions are normal within any cluster, but it is noteworthy how variable the BL and DL distributions are in any of them. One cluster (referred to here as cluster 4) consists of the *wee* (and semi-*wee*) sized cells with such a low number of cells that it is unsuitable for statistical analysis. The G<sub>1</sub>/S size control mechanism working in *wee1* mutants seems to operate in these cells (Svecizer et al., 1996). All the other cells (clusters 1–3) have at least WT size or are usually even much larger, meaning that for some unknown reason this G<sub>1</sub>/S size control cannot keep the great majority at the *wee* size.

Most cells belong to cluster 1 (Table 2), whose cycle time is very close to that of the so called minimal cycle of fission yeast (Fantès, 1977; Miyata et al., 1978a). The DL/BL ratio is only 1.4, clearly showing that these cells do not double in size during their cycle, therefore the size must decrease in the successive generations. Consistent with this, the total extension does not depend on BL, indicating the lack of size control. The mean cycle time in cluster 2 is close to those of WT cells (Table

**Table 2. Analysis of the *wee1-50 cdc25Δ* double mutant**

1. Cluster	2. No. of cycles analysed	3. BL (μm)	4. DL (μm)	5. CT (min)	6. CofV <sub>CT</sub> (%)	7. CofV <sub>DL</sub> (%)	8. Slope Ext/BL (μm/μm)	9. Slope CT/BL (min/μm)
1.	246	11.3 <sup>1.76</sup>	15.5 <sup>2.02</sup>	93 <sup>10</sup>	10.8	13.0	0.03 <sup>(NS)</sup>	-3.1 <sup>(S)</sup>
2.	175	10.4 <sup>1.36</sup>	19.1 <sup>2.25</sup>	167 <sup>13</sup>	7.8	11.8	0.37 <sup>(S)</sup>	-4.1 <sup>(S)</sup>
3.	30	9.4 <sup>1.13</sup>	22.9 <sup>2.81</sup>	242 <sup>17</sup>	7.0	12.3	0.17 <sup>(NS)</sup>	-5.4 <sup>(S)</sup>
4.	14	5.7 <sup>0.69</sup>	8.6 <sup>0.72</sup>	139 <sup>23</sup>	16.6	8.4	-	-
1.+2.+3.+4.	465	9.9 <sup>1.96</sup>	17.9 <sup>3.72</sup>	132 <sup>47</sup>	35.6	20.8	-	-

For the clusters, see text. Columns 3-5, mean values + s.d. as superscript. BL, birth length; DL, division length; CT, cycle time. Columns 6-7, coefficient of variation (CofV) of the indexed parameter. Columns 8-9, slopes of the indicated graphs (Ext, total extension). Superscripts give the significance (S) or not (NS) of the slope deviating from zero. The slopes are not given for cluster 4 because of very few observations, and also not given for the total population because of the clustered behaviour.

2). Furthermore, The DL/BL ratio is 1.8 which is close to doubling in size, considering that cells round off at division. On the other hand, there is no negative correlation between Ext and BL, so size control does not work in this group either. Cluster 3 has only a few cells with an extremely long cycle time (Table 2) during which they reach 2.4 times their birth lengths, meaning a continuous drift to the larger and larger values. Size control seems to be missed again.

To summarise our results above we argue that some cells are *wee* sized in which the G<sub>1</sub>/S size control acts. Most cells are larger, therefore the G<sub>1</sub>/S control becomes cryptic. A mitotic size control should work here, but it does not happen because of the much reduced *wee1* activity. So, the cycle time splits but homeostasis is not maintained in any of the clusters. The more interesting fact is that in spite of this deficiency, this strain is viable and does not even have a high mortality rate during growth. Furthermore, the average size of the whole culture does not seem to change in time (data not shown), as would be expected in the absence of size control (Fantès and Nurse, 1981). So, there is the strange case here that a population maintains size homeostasis without size control.

Analyses of sisters and mother-daughter pairs are given in Table 3. Surprisingly, sister cells usually behave differently (Table 3A), so that in more than half of the observations the sisters do not belong to the same cluster. The only exception is cluster 4, when the *wee* sized cells seem to have similar sisters. Although division is a bit more asymmetric in this mutant compared to WT cells, but less asymmetric than in simple *wee1* mutants (data not shown), so this phenomenon is not caused by an imprecise septum deposition. Since no rule could be found describing the difference in sisters we assume that belonging to a cluster is not determined at cell birth and it may rather change later in the cycle.

Consistent with the former result, belonging to a cluster is not an inheritable characteristic of the cell. Instead, in most cases the daughter cell seems to behave differently from its mother (Table 3B). Very often a short cycle is followed by a long one and vice versa, thus the cell line might avoid a continuous drift to either the smaller or the larger size. The only rule is that a *wee* size mother cell produces *wee* size progenies. However, in some cases cells from cluster 1 with the minimal cycle time shift to *wee* size (cluster 4, Table 3B).

We interpret the viability of this double mutant in the following way. The cells move randomly from one cluster to another in the successive generations. Although size homeostasis is not maintained in any cluster (with the

exception of the *wee* sized cells in cluster 4), this random movement causes a quasi-homeostasis in the total population, since it more or less effectively prevents a cell becoming extremely large or small. However, sometimes both events might occur, but the population consists of few *wee* and *cdc* phenotype cells only. The vast majority maintain the quasi-homeostasis of size by random movements among clusters 1-3.

#### (2) Patterns of length extension in the *wee1-50 cdc25Δ* mutant

In a further study of the physiology of this mutant, we analysed growth patterns of many individual cells from three of the above discussed four clusters.

In cluster 1, the average cycle time is made up from a 58 minute growing period (denoted here by T<sub>1</sub>) and a 31 minute constant length stage (Table 4). In ~80% of the observations, the growing period is not broken by any RCP, it consists of only one linear segment (one sample is shown in Fig. 3A). In the remaining cases, a weak RCP was observed. It is noteworthy that large *cdc2* cells after a block and release experiment, whose cycle time was close to the minimal value, also showed no RCP in the cycle (Sveiczzer et al., 1996).

A characteristic example from cluster 2 is shown on Fig. 3B, with an interesting pattern having an inner plateau (or at least a slowing down) during the growing period. This novel pattern

**Table 3. The effect of the mother and sister cells on the behaviour of a cell (*wee1-50 cdc25Δ* double mutant)**

	1.	2.	3.	4.
A S1\S2				
1.	63	60	7	0
2.	-	34	8	0
3.	-	-	4	0
4.	-	-	-	4
B M\D				
1.	41	51	19	2
2.	56	29	1	0
3.	6	1	0	0
4.	0	0	0	7

(A) The number of cases when one sister (S1) belonged to the i-th cluster (where i=1,2,3,4) while the other sister (S2) belonged to the j-th cluster (where j=1,2,3,4). The data are symmetric for the diagonal, therefore half of the table is empty. The total number of observations is 180.

(B) The number of cases when the mother cell (M) belonged to the i-th cluster (where i=1,2,3,4) while the daughter cell (D) belonged to the j-th cluster (where j=1,2,3,4). The total number of observations is 213.

**Table 4. Characteristic features of the growth pattern of the fission yeast *wee1-50 cdc25Δ* double mutant**

1. Cluster	2. T1 (min)	3. T2 (min)	4. T3 (min)	5. T4 (min)	6. T5 (min)	7. CL (min)	8. Number and ratio of cells with no inner plateau	9. Number and ratio of cells with 1 inner plateau	10. Number and ratio of cells with 2 inner plateaus
1.	58	–	–	–	–	31	52 (100%)	0 (0%)	0 (0%)
2.	61	13	64	–	–	31	6 (11%)	48 (89%)	0 (0%)
3.	61	16	57	12	58	35	1 (5%)	4 (19%)	16 (76%)

For meaning of the clusters, see text. Columns 2-7, the mean values of T1, ..., T5 and CL, respectively (the definitions are given in the text). Since clusters 1 and 2 do not have certain properties by definition (namely, T2-T5 and T4-T5, respectively), these values are missing.

was observed in 89% of the cells (Table 4). However, in ~60% of the cases the rate of length extension does not accelerate after the slowing down, compared to the value before it (in the remaining cases a weak RCP was observed). The time spent in the first growing period is denoted by T1, the duration of the inner slowing down by T2, and of the second growing period by T3 (Fig. 3B, and mean values are given in Table 4). Note that T2 is less than half of the duration of the CL stage, and that although T3 is a bit larger than T1, the slowing down cuts the growing period into two parts more or less symmetrically.

In cluster 3, most cells (from the observable 21 ones) had two inner plateaus, therefore the growing period is divided into five parts, denoted by T1, ..., and T5, respectively (Fig. 3C; Table 4). Observe that the mean T1, T3 and T5 values are very similar and T2 is also close to T4, the latter ones being less than half of CL again. Typically, there is at least one weak RCP during such a long cycle, i.e. after one (or both) slowing down(s) the rate of length extension increases but does not double (data not shown). In four cases we noticed only one plateau during the growing period (Table 4). In spite of so few observations, it is interesting that in all these cells this single slowing down cuts the growing period into two parts asymmetrically, in a different way from cluster 2. In three cases it occurred at ~1/3 and in one case at ~2/3 of the growing period, suggesting that the other slowing-down could not be detected. Finally, we observed only one cell having no inner plateaus at all. So, the number of inner plateaus in the 21 cases was 36, that is, 86% of its maximal value (42, if all cells had two such slowing downs). Remember that in cluster 2, 89% of the cells had one slowing-down (Table 4), so the not too rare omission of the temporary cessation of growth in the cycle seems not to be accidental.

The general view of our results is that after birth this double mutant grows for ~60 minutes and then length extension stops. There are two possibilities here. The cell might complete its normal cycle after a ~30 minute CL stage (cluster 1). Alternatively, after a ~15 minute plateau it could return to an earlier stage of the cycle and start to generate a new one without finishing the previous one (cluster 2 and 3). The main questions here are: (a) which event aborts during the unfinished cycles and why? and (b) which stage does the cell return to?

Some cytological observations from the films were made on cells with the longest cycles (cluster 3). Cells were never found with multiple septa. The majority of cells had one nucleus and only a minority had two. No cell had more than two nuclei, even at the end of their cycles. This is important since Mitchison and Nurse (1985) also observed inner plateaus during the block of the early septation mutant, *cdc11*, but those cells became multinucleated and the length of the slowing

down was similar to that of CL. So, the *wee1-50 cdc25Δ* mutant could not complete mitosis, at least could not execute anaphase during this inner plateau. In a normal cycle, growth stops during the early events of mitosis, when actin moves from the tips to the middle of the cell to make a contractile actin ring needed for septum formation (Chang and Nurse, 1996). All these results suggest that during the slowing-down period the cells from cluster 2 and 3 aborted mitosis and returned from an early mitotic stage (probably metaphase).

The next question might be if there is any DNA re-replication or not, i.e. are these cycles endoreplicative ones? Although the DNA content of the cells was not measured, we can in practice exclude this possibility. There were no signs of the enlarged nuclei which have been observed in other situations during endoreplication (Creanor and Mitchison, 1990; Moreno and Nurse, 1994; Yamaguchi et al., 1997). If this mutant were re-replicating its DNA during the long cell cycles (cluster 3) then the DNA content would increase 8-fold. Re-replication would result in a steady increase in the ploidy of the culture, which can be ruled out.

All our results described above suggest 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 G<sub>2</sub> and starts again preparing for mitosis. Earlier we argued that cycle time is quantised in these cells (Svecizer et al., 1996), but this statement needs to be refined now. Instead of executing the total cycle without division more times, these cells pass only the G<sub>2</sub> and part of the M phases repeatedly, but neither re-replicate nor finish nuclear division meanwhile. Since anaphase and the G<sub>1</sub>/S phases are short in a normal cycle, and furthermore, G<sub>1</sub> becomes even shorter in large cells (Novak and Mitchison, 1990), the cycle times are nearly doubled. However, one can observe that the mean cycle time in cluster 2 is less than twice the mean cycle time in cluster 1, and so on (Table 2). This difference correlates well with the fact that the inner plateaus are considerably shorter than CL (Table 4).

## DISCUSSION

### Mitotic size control in the *cdc25Δ* genetic background

The classical physiological studies of size control in *S. pombe* in the 1970s led to a conclusion that it should act at the end of G<sub>2</sub> in WT, so G<sub>2</sub> was a 'timer' followed by a 'sizer' (Nurse and Fantes, 1981). Twenty years later we concluded that the old model was incorrect (Svecizer et al., 1996). The mitotic size control acted in the middle of G<sub>2</sub> and G<sub>2</sub> was rather a

'sizer' followed by a 'timer'. Although the mechanism of mitotic size control is not fully understood, it might work in principle either by inhibitor (*wee1*) dilution (Wu et al., 1996), or by activator (*cdc2* kinase) accumulation in the nucleus (Booher et al., 1989; Novak et al., 1998). Irrespective of which mechanism is operating, at a critical cell size in the early G<sub>2</sub> phase *wee1* is no longer able to block the progress of cell cycle events. At this point molecular preparations for mitosis begin and MPF kinase activity starts to rise (Creanor and Mitchison, 1994). This might also cause a partial re-arrangement of the cytoskeleton in the cell, leading to NETO and therefore an RCP.

The connection between kinase activity and re-assembly of the cytoskeleton is known in *Saccharomyces cerevisiae* (Lew and Reed, 1995), but there is no evidence in *S. pombe* although it has been suggested for many years (Robinow and Hyams, 1989; Gould and Simanis, 1997; Verde, 1998). Thus the rate-limiting step during the long G<sub>2</sub> timer is possibly the activation of MPF by Tyr15 dephosphorylation of its *cdc2* subunit. Since the main enzyme responsible for this process is *cdc25*, our assumption could be improved by examining mitotic size control in the total absence of *cdc25* activity.

In this paper it has been shown that the mitotic size control in *cdc2-3w cdc25Δ* cells acts in late G<sub>2</sub> which is different from WT, although its position could not be determined precisely. Surprisingly, this resembles the old model of size control in WT (Nurse and Fantes, 1981). The simplest explanation is that if *cdc25* is responsible for the late G<sub>2</sub> timer in WT, then in the absence of this mitotic inducer this timer should be infinite; but in mutants not requiring *cdc25*, this timer should be reduced or perhaps eliminated. How then does the size control operate in the absence of *cdc25*?

We suggest that *cdc2-3w* cells are able to tolerate the total absence of *cdc25* (and even its back-up, *pyp3*) activity because this dominant hyperactive allele encodes a protein having a considerable MPF activity in complex with *cdc13* even if the *cdc2* subunit is Tyr15 phosphorylated. As a consequence the rate limiting step in the initiation of mitosis might be the formation of *cdc2/cdc13* complexes or rather their transport from the cytoplasm to the nucleus instead of the Tyr15 dephosphorylation of *cdc2*. In WT, the level of *cdc13* starts to increase at ~0.3 cell cycle position (Creanor and Mitchison, 1996) which could also occur in this mutant. This accumulation might depend on cell size since larger cells can synthesise *cdc13* faster. The key size requirement could be the accumulation of a critical kinase activity in the nucleus in late G<sub>2</sub>, close to the G<sub>2</sub>/M transition.

### Quantised G<sub>2</sub> timer in the absence of both *wee1* and *cdc25*

The other situation where a cell survives the total lack of *cdc25* is the *wee1-50 cdc25Δ* double mutant when the main Tyr15 kinase and phosphatase are both absent. This cell needs the *pyp3* back-up phosphatase (Millar et al., 1992), probably because Tyr15 is phosphorylated by the back-up kinase, *mik1* (Lundgren et al., 1991). Recently we noticed that these extremely variable cells can be grouped into clusters based on their cycle times (Sveiczner et al., 1996); a phenomenon which had never been found so far in *S. pombe*, including in *wee1-6 cdc25-22* (Fantes, 1979). This latter mutant probably has some residual *cdc25* activity even at 35°C causing a *wee* phenotype;

so we conclude at present that the behaviour of cycle time quantisation may occur only in the total absence of *cdc25* activity.

We showed here that mitotic size control did not operate in any cluster of the *wee1-50 cdc25Δ* mutant, so except for the few *wee* sized cells this population could not maintain size homeostasis. The contradiction that this mutant is viable was explained by the observation of random movement of the cells from one cluster to another in successive generations, providing quasi-homeostasis to the whole culture. To our knowledge, this is the first published case where a population is viable and long-lived in the absence of size control.

We have given a detailed description of the quantised cell cycles found in our earlier paper (Sveiczner et al., 1996). Cytological observations as well as the cycle time distributions indicate that very often a cell aborts mitosis at an early stage and returns back to the beginning of G<sub>2</sub>. Cessation of length growth is probably caused by actin movement from the tips to the middle of the cell to form the medial ring, an event generally occurring sometime around metaphase, but not positioned more exactly (Chang et al., 1996). The length of this inner plateau could be a function of the point the cell returns from, or the cell could return from the same point in every case but the speed of actin movement might differ. If the cell aborts mitosis, actin probably returns to the tips along microtubules and this re-movement might be determined by the *tea1* protein which is always detectable at the poles and has an important role in the morphogenesis of fission yeast (Mata and Nurse, 1997).

We suggest that the *wee1-50 cdc25Δ* double mutant cell is able to abort mitosis because it cannot initiate anaphase. Probably the cell has sufficient MPF activity to start mitosis but not to turn on APC (anaphase promoting complex) necessary for starting anaphase (Nasmyth, 1995). Because H1 kinase activity peaks at the onset of anaphase (Creanor and Mitchison, 1994), it probably increases during early mitosis, so returning from metaphase because of insufficient MPF activity might be a real possibility.

In support of this hypothesis, the largest cycling *wee1-50 cdc25Δ* cells (14 μm < BL ≤ 17 μm; possibly with the highest H1 kinase activity) always exit from mitosis after the first entry and they have a short cycle time (see Fig. 6A of Sveiczner et al., 1996). However, the smaller cells often cannot complete mitosis properly and go back to early G<sub>2</sub> phase, which suggests that they must lose even their earlier MPF activity. The reason for this is not known at the moment, though it is likely to be connected to Tyr15 phosphorylation. Once a cell loses its MPF activity in metaphase, possibly by Tyr15 phosphorylation, and starts preparing for mitosis from the beginning, it will forget its former life and its future will be independent of its past, and of the behaviour of its mother and sister cells.

In the absence of *cdc25* and *wee1*, the length of the cycle, the onset and the abortion of mitosis might be determined by the back-up enzymes, *mik1* and *pyp3*. The regulation of these enzymes during the cycle is not known and we can only speculate that the balance of their activities changes during mitosis to promote Tyr15 phosphorylation and exit from mitosis. In the presence of *cdc25* this effect cannot be manifested since *cdc25* activity is dominant over these back-up enzymes. To test this hypothesis biochemically would be an interesting problem in the future, just like finding more

situations with this strange behaviour in which a population splits into clusters, as has also been found in another organism *Paramecium* (Kippert, 1996). Very recently a similar phenomenon has also been published by Ye et al. (1998): rapid inactivation of *bimA* in *Aspergillus* causes periodic chromosome condensation and decondensation.

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