GROWTH IN CELL LENGTH IN THE FISSION YEAST

Schizosaccharomyces pombe

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SUMMARY

The cylindrical cells of Schizosaccharomyces pombe grow in length by extension at the ends and not the middle. At the beginning of the cell cycle, growth is restricted to the ‘old end’, which existed in the previous cycle. Later on, the ‘new end’, formed from the septum, starts to grow at a point in the cycle that we have called NETO (‘new end take-off’). Fluorescence microscopy on cells stained with Calcofluor has been used to study NETO in size mutants, in blocked cdc mutants and with different growth temperatures and media. In wild-type cells (strain 972) NETO happens at 0-34 of the cycle with a cell length of 9-5 μm. With size mutants that are smaller at division, NETO takes place at the same size (9-0–9-5 μm) but this is not achieved until later in the cycle. Another control operates in larger size mutants since NETO occurs at the same stage of the cycle (about 0-32) as in wild type but at a larger cell size. This control is probably a requirement to have completed an event in early G2, since most cdc mutant cells blocked before this point in the cycle do not show NETO whereas most of those blocked in late G2 do show it. We conclude that NETO only happens if: (1) the cell length is greater than a critical value of 9-0–9-5 μm; and (2) the cell has traversed the first 0-3–0-35 of the cycle and passed early G2.

NETO is delayed in poor media, in which cell size is also reduced. Temperature has little effect on NETO under steady-state conditions, but there is a transient delay for some hours after a temperature shift. NETO is later in another wild-type strain, 132.

Time-lapse photomicrography was used to follow the rates of length growth in single cells. Wild-type cells showed two linear segments during the first 75% of the cycle. There was a rate-change point (RCP), coincident with NETO, where the rate of total length extension increased by 35%. This increase was not due simply to the start of new-end growth, since old-end growth slowed down in some cells at the RCP. cdc11.123 is a mutant in which septation and division is blocked at 35°C but nuclear division continues. The constant-length stage, which occupies the last 25% of the cycle in wild-type cells, also occurs in this mutant at 35°C, so end growth stops and restarts even though there is no septation. In cdc2.33, a mitotic mutant, there is a linear pattern with an RCP in cells held at 35°C. The RCP is therefore a periodic event that can take place even though the DNA division cycle is blocked.

INTRODUCTION

The growth in cell length during the cell cycle of Schizosaccharomyces pombe has been examined with interference microscopy (Mitchison, 1957), fluorescence

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microscopy (May, 1962; Streiblova & Wolf, 1972) and autoradiography (Johnson, 1965). There is agreement that the cylindrical cells grow only at the ends and not in the middle, and that growth ceases during the constant-length stage in the last quarter of the cycle. There is also agreement that the smaller cells early in the cycle grow only at the 'old end', which is not formed from the septum at the preceding cleavage. Later in the cycle, cells start into growth at the other 'new end', so both ends expand. There is some disagreement about the proportion of cells growing at both ends, and the shape of the growth curves has not been followed in fine detail.

We have used fluorescence microscopy and time-lapse photomicrography to carry out an extensive study of this subject not only on wild-type cells but also in size mutants and cdc mutants. We have found that cell size is an important factor in initiating growth at the new end. We have also found that the growth patterns are linear with a point of rate change in the first half of the cycle. Rate changes persist after cdc blocks to mitosis and to septum formation.

**MATERIALS AND METHODS**

**Strains**

The strains used are listed in Table 1 together with their phenotypes and a reference describing their isolation. The cdc mutants are all temperature-sensitive lethals, which become blocked at various stages of the cell cycle when incubated at their restrictive temperature of 36 °C. The exception is cdc2-1w, a wee mutant, which divides at a small size. This is phenotypically similar to wee1-50 and wee1-1. All strains used were heterothallic in mating type, being h + or h – and were checked to ensure they were haploid before use.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>972</td>
<td>Wild type</td>
<td>Leupold (1950)</td>
</tr>
<tr>
<td>132</td>
<td>Wild type</td>
<td>N.C.Y.C. 132 N.S. (Mitchison &amp; Creanor, 1971)</td>
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<tr>
<td>cdc1-7</td>
<td>G2 block</td>
<td>Nurse, Thuriaux &amp; Nasmyth (1976)</td>
</tr>
<tr>
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<td>G2 block</td>
<td>Nurse et al. (1976)</td>
</tr>
<tr>
<td>cdc2-lw</td>
<td>Small size</td>
<td>Nurse &amp; Thuriaux (1980)</td>
</tr>
<tr>
<td>cdc6-23</td>
<td>G1 block</td>
<td>Nurse et al. (1976)</td>
</tr>
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<td>G1 block</td>
<td>Nurse et al. (1976)</td>
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<td>cdc11-123</td>
<td>Septation block</td>
<td>Nurse et al. (1976)</td>
</tr>
<tr>
<td>cdc13-117</td>
<td>M block</td>
<td>Nurse et al. (1976)</td>
</tr>
<tr>
<td>cdc17-K42</td>
<td>DNA ligase defect</td>
<td>Nasmyth (1977)</td>
</tr>
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<td>cdc20-M10</td>
<td>G1 block</td>
<td>Nasmyth &amp; Nurse (1981)</td>
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<tr>
<td>cdc22-M45</td>
<td>G1 block</td>
<td>Nasmyth &amp; Nurse (1981)</td>
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<td>cdc25-22</td>
<td>G1 or M block</td>
<td>Thuriaux, Sipiczki &amp; Fantes (1980)</td>
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<td>Small size</td>
<td>Nurse &amp; Thuriaux (1980)</td>
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<tr>
<td>wee1-50</td>
<td>Small size</td>
<td>Nurse (1975)</td>
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</table>
Growth in cell length in S. pombe

Media and growth conditions

The media used were based on a modified minimal EMM2 medium (Nurse, 1975) supplemented with 0.5% (w/v) Difco yeast extract. For certain experiments the yeast extract supplement was not added and 10 mM-proline was used as the nitrogen source replacing the NH₄Cl in the minimal medium. Malt extract broth (Oxoid) was used at 2% (w/v). Cultures were grown with shaking in a water bath, usually at 25°C but for certain experiments at 32°C, 35°C and 36°C.

Fluorescence microscopy

The procedures used were essentially similar to those described by Streiblova & Bevan (1963). Cells were grown to mid-exponential phase (2x10⁶ to 5x10⁶ cells/ml), 1 ml was centrifuged, washed with 1 ml ice-cold 1% saline, and resuspended in 100 µl of ice-cold saline containing 1 mg/ml Calcofluor (American Cyanamid). The cells were kept on ice for 10–15 min and were examined with either a Vickers or Zeiss fluorescence microscope (×40 and ×100). Cell lengths were measured using either an eyepiece scale micrometer or a drum eyepiece micrometer.

The Calcofluor staining reveals whether cells are growing at one end or two, and by counting the numbers of cells in each class an estimate can be made of the time in the cell cycle when growth begins at both ends (see Results). After cell division cells hang together for a short while before undergoing final separation. As a consequence the calculated cell cycle timings will be related to final separation rather than division. To correct for this, populations of cells were subjected to brief sonication, which disrupts pairs of cells that have completed division but not separation. Examination of these populations showed that there was a delay of 0.12 of a cell cycle between division and separation, and this correction has been applied to all the estimates given in the paper. Therefore all cell cycle timings relate to cell division after the septum has been completed rather than to final cell separation.

Time-lapse photomicrography

For the time-lapse films of strain 972, the cells were taken from growing cultures in early exponential phase in the modified EMM2 medium supplemented with 0.5% (w/v) yeast extract. They were then mounted in a chamber between a coverslip and a pad of the same medium plus 2% agar. Warm water was circulated round the perimeter of the chamber and in some cases the temperature in the agar was monitored by a thermistor. The chamber was placed on the stage of a Zeiss photomicroscope surrounded by a hot box heated by an Accutron air circulator. The cells were photographed every 5 min on Ilford Pan F film using a bright field ×25 planapochromatic objective (N.A. 0.65). Measurements were made with a ruler either on projected images or on enlargements from the negative. With these optical conditions, it was almost always possible to detect the division scars.

This technique did not work satisfactorily with cdc mutants held at the restrictive temperature, since many of the cells died when sandwiched between the agar and the coverslip. This problem was overcome by introducing a 1–2 mm air gap between the cells on the agar and the coverslip. The optical image, however, was worse both because of the air/water interface round the cells and because it was necessary to use an objective of longer working distance and lower resolution (×16 achromat, N.A. 0.32). As a result, it was only possible to detect the division scars in a minority of cells. With cdc mutants, the cells were mounted in the chamber and grown at the permissive temperature for 3 h before the temperature was raised to the restrictive level. The change in temperature took less than 5 min.

RESULTS

Fluorescence microscopy

Division scars and patterns of cell growth. Two wild-type fission yeast cells stained with Calcofluor are shown in Fig. 1. The most prominent feature is the brightly
stained septum crossing the middle of the dividing cell I. But also visible are the birth or division scars, which mark the position of a previous septum site. These are seen as dark lines crossing the cells, two on cell I and three on cell II. They are formed when a cell divides in two; the brightly staining primary septal material falls away and leaves a dark division scar. The scar forms a darkened hemisphere at the new end of the daughter cell as seen at the tip of cell II. The darkened hemisphere remains until the cell begins to grow from its new end. Then the division scar is pushed back behind the newly synthesized cell wall material to form a dark ring around the cell. This has taken place in cell I. Therefore, it is possible to determine if a cell has grown at all at its new end by observing whether it still has a darkened hemisphere as in cell II or an internalized dark ring as in cell I. The dark rings remain as permanent features of the cell wall marking the position of septation from previous cell division cycles. One such relic of previous cell cycles is seen in cell I and two in cell II. But it should be noted that only the division scar at or nearest to the tip of the cell shows whether the cell has grown at its new end or not.

An estimate of the cell cycle timing of when cell growth is initiated at its new end (called ‘new end take-off’ or NETO) can be made by counting the proportion of cells in a population that have grown at the new end. Making the reasonable assumption that those cells that have not yet undergone growth at their new end are in the first part of the cycle allows the cell cycle distribution equation (Howell, 1974) to be applied. This enables the time in the cell cycle when NETO occurs to be calculated. A total of 400 cells of a wild-type population growing at 25°C in yeast extract supplemented minimal medium were analysed in this way and the time when NETO occurs was estimated to be about 0.34 through the cell cycle. As well as estimating when in the cell

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Fig. 1. Two wild-type cells stained with Calcofluor. A brightly stained septum is seen crossing the middle of cell I and dark lines marking birth or division scars are seen crossing both cells. The inset drawing shows distance $a$ between the end of a cell and the division scar, and $b$ the total length of the cell.
cycle NETO occurs it is also possible to measure at what cell length NETO takes place. This can be done by measuring the distance between the end of a cell and the site of the division scar (distance $a$ in Fig. 1) and the total length of that cell (distance $b$ in Fig. 1). By making measurements of a large number of cells in a population it is possible by plotting $a$ versus $b$ to find the cell length at which $a$ begins to increase indicating that NETO has taken place. Such a plot based on 200 cells is shown in Fig. 2B for a wild-type population growing at 25°C in medium supplemented with yeast extract (cells were grouped in total-length classes spanning between increases in length of 0.5 μm and 0.8 μm). It can be seen that $a$ remains the same at around 1.5-2 μm until cells attain a length of about 9.5 μm. Cells that have grown beyond this length undergo NETO and as a consequence $a$ gradually increases as total cell length increases. There is a very slight increase in $a$ before 9.5 μm, but this is minor compared to the dramatic increase seen after 9.5 μm, allowing cell length at NETO to be calculated quite precisely. The values of 0.34 of a cell cycle and 9.5 μm cell length

Fig. 2. Length from birth scar to new-end tip plotted against total cell length for three strains: A, wee1.50; B, 972, wild type; C, cdc25.22. Arrows mark point of inflexion where line increases in slope. This indicates the cell length at NETO.
can be shown to be consistent with each other. Assuming a cell length at septation of 13-3 μm (Table 2) the cell length at birth can be calculated as 0.5 × 13-3 × 1.11 = 7.38 μm. This assumes the usual correction factor of ×1.11 for S. pombe cells dividing into two (Mitchison & Walker, 1959). The length increase during the cell cycle must be 13.3 μm−7.38 μm = 5.92 μm and during 0.34 of a cell cycle (assuming linear growth) must be 0.34 × 5.92 μm = 2.01 μm. Therefore, the length at 0.34 of a cell cycle will be 7.38 μm + 2.01 μm = 9.39 μm, very close to the observed value of 9.5 μm.

From the data given in the previous section it can be seen that wild-type cells begin to grow at their new end and undergo NETO at about 0.34 of the way through their cell cycle and at a length of about 9.5 μm. A general explanation might be that NETO occurs when cells attain a critical length of 9.5 μm irrespective of their length at division. In order to test this possibility a variety of wee and cdc mutants that undergo division at a range of sizes were examined. Mutants of wee1 (wee1-1, wee1-50) and wee mutants of cdc2 (cdc2.1w) divide at a smaller size than wild-type, and various cdc mutants when grown at their permissive temperature divide at a larger size than wild type. If NETO occurs at a critical cell length, then this will be later in the cell cycle in the mutants dividing at a small size, and earlier in those dividing at a large size.

The various mutants were grown in minimal medium supplemented with yeast extract at 25°C and the cells were stained with Calcofluor. At least 200 cells of each population were measured to estimate a and b as shown in Fig. 1. A plot of a versus b was then made for each population. Examples of three of the populations are given in Fig. 2. The cell length at which the inflexion took place, corresponding to NETO, was determined for all strains (marked by an arrow in the three examples of Fig. 2). These values are tabulated in Table 2. It can be seen that in mutants smaller than wild type, cell length at NETO is constant at 9.0–9.1 μm, increasing slightly in wild type to 9.5 μm. Therefore, in cells of wild-type size or below, attainment of a critical cell length appears to determine when NETO takes place. However, in cells greater in size than wild type the relationship fails and cell length at NETO gradually increases as cells increase in size at division (Table 2).

To investigate the situation in these larger cells the cell cycle timing of NETO was determined by counting the proportion of the population that had undergone NETO.
Growth in cell length in *S. pombe*

At least 400 cells were screened for each population. The calculated cell cycle timings of NETO are given in Table 2. It can be seen that in the mutants dividing above wild-type size the cell timing of NETO is the same at 0:32–0:34. This suggests that NETO cannot move forward any earlier than about 0:3–0:35 of a cell cycle, regardless of how big cells are at division. In the cells of wild-type size or smaller where NETO occurs at a constant cell length its cell cycle timing moves later in the cycle as division size reduces. This is in accord with the critical cell-length hypothesis.

From these results we conclude that there are two requirements before NETO can take place. First, cells must attain a critical cell length of 9:0–9:5 \( \mu \)m. Second, they must have spent at least the first 0:3–0:35 of a cell cycle in the cycle. When both these conditions are met, then NETO can take place. In cells dividing at a size smaller than wild type the cell length requirement determines NETO and in cells dividing at a larger size it is the 0:3–0:35 of a cell cycle requirement that is important. This can be seen more clearly if the data are plotted as in Fig. 3. In the smaller mutants cell length at NETO remains constant, only increasing at around wild type. Conversely, the cell cycle timing is the same in the larger mutants but becomes delayed in the smaller mutants. The cross-over from one control to the other occurs in cells about 12:5–13 \( \mu \)m at division, just a little smaller than wild type. Presumably, in wild-type cells growing under these conditions NETO is controlled most of the time by the requirement to reach 0:3–0:35 of a cell cycle. But should cell length at division be slightly shorter than normal, say only 10\%, then the requirement to attain a length of 9 \( \mu \)m will come into play and will delay NETO until a little later in the cell cycle.

**Influence of cell cycle blocks on NETO.** The results presented above have established that two conditions must be satisfied before NETO can occur, the attainment of a cell length of around 9 \( \mu \)m and progress to about 0:3–0:35 through the cell cycle.

![Fig. 3. Cell length (●) and cell cycle timing (■) of NETO plotted against cell length at septation for various strains. Each point represents a single strain, reading from left to right: wee1.1, cdc2.1w, wee1.50, 972 wild type, cdc2.M63, cdc25.22, cdc2.M35.](image-url)
The second requirement suggests that some specific event of the cell cycle must be completed before NETO can occur. If this is the case then blocking this event will prevent NETO taking place even if cells have spent 0·3–0·35 in their cell cycle and have attained a length above 9 μm. To investigate this possibility a range of temperature-sensitive cdc mutants were incubated at their restrictive temperature (Nurse, Thuriaux & Nasmyth, 1976; Nasmyth & Nurse, 1981). This treatment blocks cells at a variety of positions in the cell cycle depending upon the cdc mutant used. For example, cdc17 is defective in DNA ligase and so a cdc17 mutant becomes blocked in a late stage of DNA replication and cannot progress towards mitosis when incubated at 36°C. The experiment is made more complicated as the cell cycle timing and cell length at NETO are disturbed even in wild-type cells after shift from 25°C to 36°C. After 5 h at 36°C, NETO is delayed to 0·8 of a cell cycle and a cell length of 12·5 μm.

When wild-type cells have grown for longer at 36°C (around 20 h) the cell cycle timing of NETO returns to a value of 0·33, similar to that observed at 25°C. Therefore, to investigate whether a cell cycle event as defined by a particular cdc mutant is needed for NETO it was necessary to determine whether the blocked cdc mutants had undergone NETO by the time they had attained a cell length of 12·5 μm. Eleven cdc mutants that were blocked in G1, S-phase, G2, mitosis or septation were incubated for 5 h at 36°C. By this time they had completed two generations of growth and were all over 25 μm in length, i.e. well over the critical length of 12·5 μm. The numbers of cells that had undergone NETO were counted and are shown in Table 3.

The data fall into two classes with one exception. In one class 20% or less of the cells had undergone NETO even though the cells were over twice the length at which this occurred in wild type. In this class were all the mutant strains that blocked either in G1 or S-phase and one G2-blocked strain cdc6.23. This last mutant blocks at an early stage in G2 between 0·25 and 0·35 of the way through the cell cycle. In the second class over 80% of the cells had undergone NETO. These mutant strains all blocked in G2, M or septation. The exception was cdc2.33, which blocks in both G1 and G2. These results suggest that some cell cycle event that requires completion of G1 and S-phase and the

### Table 3. Blocked cdc mutants

<table>
<thead>
<tr>
<th>Mutant strain and block point</th>
<th>Cell cycle stage of NETO at 25°C</th>
<th>% Cells undergone NETO after 5 h at 36°C</th>
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<tr>
<td>cdc10.129</td>
<td>G1</td>
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</tr>
<tr>
<td>cdc20.M10</td>
<td>G1</td>
<td>0·36</td>
</tr>
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<td>cdc22.M45</td>
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<td>0·35</td>
</tr>
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<td>cdc21.M68</td>
<td>S</td>
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</tr>
<tr>
<td>cdc17.K42</td>
<td>S</td>
<td>0·37</td>
</tr>
<tr>
<td>cdc6.23</td>
<td>G2</td>
<td>0·45</td>
</tr>
<tr>
<td>cdc1.7</td>
<td>G2</td>
<td>0·41</td>
</tr>
<tr>
<td>cdc25.22</td>
<td>G2 or M</td>
<td>0·36</td>
</tr>
<tr>
<td>cdc13.117</td>
<td>M</td>
<td>0·41</td>
</tr>
<tr>
<td>cdc11.123</td>
<td>Septation</td>
<td>0·34</td>
</tr>
<tr>
<td>cdc2.33</td>
<td>G1 and G2</td>
<td>0·35</td>
</tr>
</tbody>
</table>
Growth in cell length in *S. pombe* first part of *G*₂ phase is necessary before NETO can take place. Later in the cycle this event is completed and so late *G*₂ and *M* phase, as well as septation, blockers do not prevent NETO taking place. The event must occur in early *G*₂ since blocking at *cdc6* prevents it from taking place and the normal cell cycle timing is around 0·3–0·35 of a cell cycle during early *G*₂.

The exception was *cdc2.33*, which showed an intermediate level of numbers of cells having undergone NETO. In order to analyse this situation more fully a population of *cdc2.33* cells was shifted from 25°C to 36°C and the lengths *a* and *b* of Fig. 1 were measured at various times after the shift. As can be seen in Fig. 4, 90% of the cells fell into two classes, either undergoing no NETO (lower unbroken line parallel to horizontal axis) or undergoing NETO (unbroken line at 45°). This indicates that the shift divides the cells into two populations, one undergoing NETO and one that does not. This result is consistent with the phenotype of *cdc2.33*, which is known to have two block points, one in *G*₁ and one in *G*₂ (Nurse & Bissett, 1981). The cells blocked in *G*₁ do not undergo NETO whilst those blocked in *G*₂ have already undergone NETO. About 10% of the cells fall between these two populations (within the broken lines on Fig. 4). These represent cells that undergo NETO after about 3 h at 36°C. The ex-

![Fig. 4. Length from birth scar to new-end tip plotted against total cell length for *cdc2.33* after shift to 36°C. Each point represents an individual cell and the symbols indicate how long after the shift the cells were measured. Time after shift: (▲) 2 h; (⋈) 3 h; (●) 4 h; (○) 5 h; (■) 6 h. Unbroken lines drawn by eye through points; broken lines showing normal limits of the two major populations of cells.](image-url)
planation for this is not clear. They may be cells that are leaking past the first block point or, alternatively, they may be a class of cells that are delayed from undergoing NETO for some time due to the transient effect described above in wild type after a temperature shift to 36°C.

**Influence of media, temperature and strain on NETO.** Cells are smaller when grown at slower rates, and so it was of interest to see whether media that supported a lower growth rate would result in NETO later in the cell cycle. Minimal medium with ammonium and proline as nitrogen sources was used to test this possibility. With ammonium as a nitrogen source (generation time around 4 h) the cell cycle timing of NETO was 0·49 and with proline (around 6 h) it was 0·78. Therefore, at lower growth rates NETO is delayed to later in the cell cycle.

It has been shown that another *S. pombe* wild-type strain 132 undergoes very little growth at its new-end tip when grown on a malt extract broth medium at 32°C (Johnson, 1965). To confirm that this strain was behaving differently from our wild-type strain 972 we estimated NETO in malt extract and our normal minimal medium supplemented with yeast extract in this strain. In Table 4 it can be seen that on malt extract NETO occurred late in the cell cycle of 0·72 and on yeast extract it was at 0·5. Therefore, strain 132 has a later NETO on yeast extract than 972 (by about 0·15 of a cell cycle). Malt extract also has an effect and delays NETO by a further 0·22 of a cell cycle. It may be that the critical length is larger in strain 132 especially in malt extract.

Changing temperature had little effect on NETO in 972 grown on medium supplemented with yeast extract. NETO ranged between 0·30 and 0·34 for temperatures between 25°C and 36°C. It should be remembered that these are steady-state values and effects are seen, as noted above, in the transient period after shift from 25°C to 36°C. Although the increased growth rates seen at 36°C had no effect on the cell cycle timing of NETO they did have an effect on the relative contribution to total growth made by the new-end tip and the old-end tip. This can be calculated from plots of *a* versus *b* as shown in Fig. 2. The amount of growth at the new end can be calculated as a proportion of total growth for the cell. From a number of experiments carried out at 25°C and 36°C on minimal medium supplemented with yeast extract the ratio of new-

<table>
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<th>Strain</th>
<th>Media</th>
<th>Temperature (°C)</th>
<th>Cell cycle stage of NETO</th>
</tr>
</thead>
<tbody>
<tr>
<td>972</td>
<td>Minimal + YE</td>
<td>25</td>
<td>0·34</td>
</tr>
<tr>
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<td>Minimal</td>
<td>25</td>
<td>0·49</td>
</tr>
<tr>
<td>972</td>
<td>Minimal + proline</td>
<td>25</td>
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<td>132</td>
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<td>Minimal + YE</td>
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<tr>
<td>972</td>
<td>Minimal + YE</td>
<td>36</td>
<td>0·33</td>
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</table>

YE, yeast extract.
end growth over old-end growth was 0.7 and 25°C and 1.0 at 36°C (see also row (13) of Table 5). Therefore, at the faster growth rates seen at higher temperatures, growth at the new-end tip makes a greater contribution to the total growth of the cell.

**Time-lapse photomicrography**

The analysis of Calcofluor-stained cells was quick and efficient but it lacked a direct time dimension. We therefore used the more laborious method of analysing time-lapse films in order to measure the rates of tip growth.

**Wild-type cells (strain 972).** The majority of cells used for this analysis came from films taken by Dr P. A. Fantes, and we are grateful for the opportunity to use these films. The method of mounting the cells is described in detail by Fantes (1977) and is substantially similar to that outlined above.

Fig. 5 shows the results for two individual cells growing at 25°C. The top curve gives the increase in total length. The other two curves give the length from the

![Fig. 5. Growth in length of two single cells of strain 972 at 25°C during one cycle from cleavage to cleavage. (•) Total length; (■) length from division scar to old end; (▲) length from division scar to new end. Arrows mark points of rate change in total length growth. S, first appearance of septum; CL, cleavage. Each point is the mean of three measurements. See Table 5 for definition of abbreviations.](image-url)
division scar formed at the immediately preceding division to the old end and to the
new end, respectively. These curves represent growth at the two ends, since we have
found no case of length growth happening elsewhere in the cell. This has been checked
in cells with several division scars, which act as surface markers. The total length
curve shows a pattern of two linear segments in the first three-quarters of the cycle
with a rate-change point a little before mid-cycle. In the last quarter of the cycle,
length growth stops and the septum (cell plate) appears. A few cells, as in Fig. 5b, had
an initial lag before achieving a constant rate of elongation. Most others did not, as in
Fig. 5a. There is little or no new-end growth until NETO, which is close to the rate-
change point in total length. Thereafter, there is linear growth until the constant-
length stage at the end of the cycle. Old-end growth also shows two linear segments
with, in these two cells, a fall in rate at NETO.

There is an appreciable amount of scatter between the points, which makes it
difficult to estimate some of the features of these curves precisely, e.g. the time of
NETO in Fig. 5a. Most of this comes from slight changes in focus as the cells grow,
which alters the black and white diffraction pattern of the image of the ends. It would
not be easy to improve this with a higher aperture objective since it would have a
reduced depth of focus.

In order to detect the presence of curvature in the linear segments, a polynomial was
fitted to the data for the rising parts of the curves and the quadratic coefficient was
tested (by a t-test) for significance at the 5% level. None of the 10 segments in Fig. 5
showed significant curvature but there were two subjective elements in this sample
analysis. The first was that the rate-change points were put in by eye and each segment
was analysed separately. The second was that a minority of cells (10% of the total)
showed a short initial lag before achieving linear growth (e.g. see Fig. 5b). This lag
period was omitted from the analysis.

The collected data are summarized in Table 5. Some interesting points that emerge
are:

(a) Comparison of rows (3) and (4) shows that the coefficient of variation of the time
to NETO was nearly three times greater than the coefficient of variation of the length
of NETO. This suggests that NETO is more closely related to cell size than to time
after birth and is therefore in accord with the earlier results on the wee
mutants.

(b) Row (5) shows that there was no significant difference between the time of
NETO and the time of the rate-change point in total length. There was a large
variation, probably due to the difficulty of determining these points precisely in some
cells.

(c) Row (10) shows that growth rate in total length increased by an average of 35%
at the rate-change point. There must therefore have been an increase of a further 65%
at the start of the next cycle in the two daughter cells.

(d) Row (12) shows that on average there was a slight decline in the old-end growth
after the rate-change point, though there was a large coefficient of variation. In some
cells (e.g. see Fig. 5b), there was sharp decline in rate and in others an increase. There
was also a large variation in the rate of new-end growth (row (9)). NETO is not simply
a point at which the new-end growth is added onto a constant old-end growth.
Instead, there is partitioning of growth at the two ends that varies considerably from cell to cell. There is not, however, a significant negative correlation between the growth rates at the two ends.

Table 6 summarizes the results of the curvature tests described above. Only eight out of a total of 82 segments (10%) showed significant curvature. Within the limits of the tests, we conclude that the segments are in most cases linear.

Table 5. Growth of single cells of strain 972 at 25°C

<table>
<thead>
<tr>
<th>Units</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Coeff. of variation (s.d./m×100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Length at birth</td>
<td>μm</td>
<td>7.26</td>
<td>0.561</td>
</tr>
<tr>
<td>(2) Cycle time (cleavage to cleavage)</td>
<td>min</td>
<td>187</td>
<td>18.8</td>
</tr>
<tr>
<td>(3) Length at NETO</td>
<td>μm</td>
<td>9.82</td>
<td>0.657</td>
</tr>
<tr>
<td>(4) Time from birth to NETO</td>
<td>Fraction of cycle min</td>
<td>0.39</td>
<td>0.0748</td>
</tr>
<tr>
<td>(5) Time difference between NETO and RCP in total length</td>
<td>min</td>
<td>3.1</td>
<td>10.5</td>
</tr>
<tr>
<td>(6) Slope of total length segment from birth to RCP (=T1)</td>
<td>10⁻² μm/min</td>
<td>4.53</td>
<td>0.496</td>
</tr>
<tr>
<td>(7) Slope of total length segment from RCP to constant length stage (CL) (=T2)</td>
<td>10⁻² μm/min</td>
<td>5.67</td>
<td>0.529</td>
</tr>
<tr>
<td>(8) Slope of old-end growth from RCP to CL (OE2)</td>
<td>10⁻² μm/min</td>
<td>2.88</td>
<td>0.505</td>
</tr>
<tr>
<td>(9) Slope of new-end growth from NETO to CL (=NE)</td>
<td>10⁻² μm/min</td>
<td>2.80</td>
<td>0.570</td>
</tr>
<tr>
<td>(10) Ratio T2/T1</td>
<td>1.35</td>
<td>16</td>
<td>0.158</td>
</tr>
<tr>
<td>(11) Ratio OE2/T1</td>
<td>0.77</td>
<td>9</td>
<td>0.098</td>
</tr>
<tr>
<td>(12) Ratio OE2/OE1</td>
<td>0.95</td>
<td>11</td>
<td>0.243</td>
</tr>
<tr>
<td>(13) Ratio OE2/NE</td>
<td>1.24</td>
<td>15</td>
<td>0.370</td>
</tr>
</tbody>
</table>

Rows (6) to (12) from fitted lines.

Table 6. Significant curvature in segments of growth curves

<table>
<thead>
<tr>
<th>NS</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>13</td>
</tr>
<tr>
<td>T2</td>
<td>14</td>
</tr>
<tr>
<td>OE1</td>
<td>12</td>
</tr>
<tr>
<td>OE2</td>
<td>17</td>
</tr>
<tr>
<td>NE</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
</tr>
</tbody>
</table>

Number of segments of growth curves of single cells of strain 972 at 25°C showing significant (S) or non-significant (NS) curvature. Test is described in text. Significance level at 5%. Nomenclature of segments as in Table 5.
Mutant cdc11.123. This mutant is an 'early cell plate mutant' in which growth and (synchronous) nuclear divisions continue at the restrictive temperature but there is no septum formation or cell division. It is like a chain of uninucleate cells except that there are only two tips. It is therefore the appropriate situation in which to test whether tip growth is dependent on nuclear dosage and whether the constant-length stage occurs when there is no septum formation.

We have followed four cells after a temperature shift from 28.7 °C to 35.5 °C and they gave very consistent patterns, one of which is shown in Fig. 6. Segments of increasing growth rate were interrupted by plateaux where there was little or no growth. Although the nuclei were invisible, we believe that the plateaux correspond to the constant-length stage that occurs in the normal cycle between nuclear division and cleavage. The plateaux occurred at an average time interval of 126 min, which is close to the cycle time of 118 min in wild-type cells at this temperature and in this medium. The average length of the plateaux was 28 min or 22% of the interval between them. This is close to the 25% of the cycle spent in the constant-length stage. If this interpretation is correct, the normal control operates and switches off end growth, but

Fig. 6. Growth in length of a cell of cdc11.123 shifted from 28.7 °C to 35.5 °C at time zero. (•) Total length; (■) length from division scar to old end; (▲) length from division scar to new end.
Growth in cell length in S. pombe

no septum is formed and growth recommences at about the time that it normally
would after cleavage.

There was a low initial growth rate in Fig. 6, which then increased after each plateau
suggesting that the number of nuclei affected the rate, but the final slope of
0.117 \mu m/min was not significantly larger than the that of 0.101 \mu m/min for the
uninucleate blocked cells of cdc2.33 (Table 3). These changes in rate occurred mostly
at the old end, since there was little new-erfd growth. It was not possible to determine
whether there was a rate change within the growth sections.

Cells do not double in length between plateaux. In Fig. 6, for example, there was
only a 68% increase between the two plateaux. Measurements of cell diameter (in
three places) showed that there was a small amount of swelling – 2% per hour in cross-
sectional area – but this was not enough to give a doubling in volume between
plateaux.

Mutant cdc2.33. This mutant at the restrictive temperature shows a more complete
block than cdc11.123. Not only are septum formation and division blocked but also
mitosis and DNA synthesis. The DNA division cycle is stopped but growth contin-
ues. The most interesting questions here are whether and when NETO occurs
(which has been considered earlier) and also whether there are periodic events such as
rate changes that continue after the block.

The pattern of growth in total length after a block is shown for two cells of cdc2.33
in Fig. 7. There was an initial lag of about 30 min during which the cells grew slowly or
not at all. This may have been due to the cells reaching the constant-length stage.
There was then a middle section of rapid growth made up of two linear segments with
a rate-change point, as in wild-type cells. This lasted for 5 h in one of the cells. Finally,
there was a period of tail-off in which the rate of growth declined and nearly stopped.
The time at which this tail-off started was very different in the two cells. The four
linear segments in Fig. 7 showed no significant curvature when analysed by the sta-
tistical \( t \)-test.

Although this growth pattern was shown in the majority of the cells, there was a
much greater variation than in wild-type cells. This is not altogether surprising since
ultimately all the mutant cells stop growing and die when maintained at the restrictive
temperature. Some of the cells had a long lag and an early tail-off, so the middle sec-
tion was too short to show two linear segments. In others, there was only a single linear
segment, which could have been due to an undetectably small increase at the rate-
change point. NETO provides a guide to the position of the rate-change point in wild-
type cells, but this could not be used with the mutants because the division scars were
not visible in most of the cells under the optical conditions used. In any case, there is
evidence given below that NETO and the rate-change point are not necessarily
associated in blocked mutants. For these reasons we have not included 10 out of the 28
cells analysed in the collected data in Table 7.

The rate increase of 33% at the rate-change point (row (7) of Table 3) is very
similar to that of 35% in wild type (Table 5), but is must be an overestimate since
patterns that may have had very small increases would not have been included in the
data.
The most interesting point in Table 7 is that the coefficient of variation of time to the rate-change point is twice as large as that of length at the rate-change point. As with wild-type cells, it suggests a closer association of the rate change with size rather than with time.

The information about ends is scanty because it was only possible to define the division scar in a few cells. In three cells, however, it was clear that there was no correlation between NETO and the rate-change point. In two of the cells, there was a rate change in the old-end growth without any new-end growth. In the third, NETO happened at a different time from the rate change. So the mutant block appears to allow dissociation between two events, NETO and the rate change, which are closely coupled in normal growth.
DISCUSSION

These results clearly demonstrate that NETO, the point in the cell cycle when the new end starts to grow, is under two controls. First, cells must attain a critical length of 9-0–9.5 μm; and second, they must have completed some event in early G2. If either of these two requirements are not met then NETO cannot take place. In cells that divide at a small size NETO is delayed until cells attain the critical length, and in large dividing cells NETO is dependent upon the completion of the event in early G2. Such a system is reminiscent of the 'sizer' and 'timer' model proposed for determining the timing of S phase in S. pombe (Nurse & Thuriaux, 1977; Fantes & Nurse, 1978). According to this model, DNA replication is only initiated when cells attain a certain critical cell size and when they have completed a short G1 period of about 0.1 of a cell cycle. In small dividing cells such as the wee mutants, S phase is delayed until later in the cell cycle when the cell attains the critical size. In large dividing cells S phase is initiated after the short 0.1 of a cell cycle G1 period. The controls, therefore, of both S phase and NETO have the same two components, critical size and traverse of a part of the cycle. There is also a dependency relation since NETO does not occur until S phase is completed. It is possible that there is a closer relationship between the two control systems. For instance, the cell cycle timing of the early G2 event upon which NETO is dependent could be determined by the same control system that operates over the timing of S phase. In this case NETO would occur at a certain fixed period after the completion of S phase. However, a problem with this interpretation is that there is a long gap between S phase and NETO, which is usually at least 0.5 of a cell cycle in wee mutants (calculated from the S-phase timings given by Nasmyth, Nurse & Fraser, 1979).

The influence of cell size on the timing of cell cycle events has been studied for a number of other processes in S. pombe. The timing of a rate doubling in messenger RNA synthesis is correlated with cell size (Fraser & Nurse, 1978, 1979), but no correlations were observed for the patterns of the synthesis of ribosomal RNA (Elliott, 1983) or total protein (Creanor & Mitchison, 1982). Steps in enzyme potential for

<table>
<thead>
<tr>
<th>Table 7. Growth of single cells of cdc2.33 at 35.4°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Size at birth (most at 28.2°C)</td>
</tr>
<tr>
<td>(2) Length at RCP</td>
</tr>
<tr>
<td>(3) Time from temp. shift to RCP</td>
</tr>
<tr>
<td>(4) Slope at 28.2°C</td>
</tr>
<tr>
<td>(5) Slope at 35.4°C; first linear segment</td>
</tr>
<tr>
<td>(6) Slope at 35.4°C; second linear segment</td>
</tr>
<tr>
<td>(7) Ratio of slopes at RCP</td>
</tr>
</tbody>
</table>
sucrase and arginase are later in the cycle for small mutants than for wild-type cells and earlier for large mutants, but the extent of these changes is not enough to support a model of control by critical cell size (Benitez, Nurse & Mitchison, 1980).

The pattern of cell elongation during the cell cycle is rather complex. We have confirmed earlier studies that demonstrated that growth stops for the last quarter of the cycle. Growth during the first three-quarters begins with a linear segment, although a few cells show an initial lag. There is then a rate-change point followed by a second linear segment, which persists until the 'constant-length stage' starts at the time of mitosis. In wild-type cells the RCP is coincident with NETO. Thus the increase in overall elongation rate of the cell is usually associated with the switching on of growth at the new end. However, this is not simply due to adding new-end growth onto a constant rate of old-end growth. In many cases old-end growth actually slows down after NETO and the ratio of growth rates of the two ends is very variable. On average, after the RCP, the total cell-elongation rate increases by 35%. Both ends stop growing at mitosis and cell wall growth shifts from the ends to the centre of the cell where the septum is formed. After cell division the sites of wall growth shift back from the septum to the two old ends and the overall rate for the two daughter cells together doubles compared to the rate at the beginning of the cell cycle of the original mother cell. Nothing else studied so far in this yeast shows such complex behaviour in growth pattern, although linear patterns with a doubling at a RCP have been found for total dry mass (Mitchison, 1957), to messenger RNA (Fraser & Moreno, 1976), CO₂ production (Creanor, 1978a) and enzyme activity (Mitchison & Creanor, 1969).

Growth continues in many cell systems when the DNA division cycle is blocked by inhibitors or by the use of cdc mutants, but it might well be assumed that the periodicities in normal growth would be abolished when the periodic events of the DNA division cycle were stopped. Such indeed is the case in S. pombe for ribosomal RNA synthesis (Elliott, 1983) and total protein synthesis (Creanor & Mitchison, 1984). But this is not so with O₂ consumption (Creanor, 1978b), CO₂ production (Creanor, 1978a) and enzyme potential (Benitez et al. 1980), where periodic rate changes persist after a block. Similar results in other cellular systems have been reviewed by Mitchison (1984). Our results with cdc2.33 provide another interesting example of a persistent periodic event, since there is a RCP at about 3 h after transfer to the restrictive temperature. As in some of the other measurements, it is possible to detect only one periodicity, since growth becomes limited or tails off after a long blockade. We do not know the basis of these periodicities but a size-control is a possibility, with the signal being given by the attainment of twofold multiples of cell size. It may be significant that the average size at the RCP is 19.4 μm, very nearly twice the length of 10.3 μm, which can be calculated from Tables 3 and 7 to be the size at NETO of the mutant cells at the permissive temperature. Further data, however, would be needed to confirm this proposal.

The 'early cell plate' mutant cdc11.123 at the restrictive temperature is blocked only in septation and not in the other two events of the DNA division cycle, DNA synthesis and mitosis. Assuming that we are correct in concluding that the growth plateaux occur after each mitosis, the most important aspect of this mutant is that the normal
Growth in cell length in *S. pombe*

morphogenetic change, from tip growth to no growth during the constant-length stage and then to renewed tip growth, can occur without the formation or splitting of a septum. It is also interesting that growth rate increases with nuclear number (though not proportionately). A final point is that both this mutant and also *cdc*2.33 show dissociation between NETO and the RCP, events that are closely coupled in wild-type cells. In several cases, rate changes occurred without any new-end growth.

The basis of the rate change in cell elongation is an interesting but mysterious problem. The data suggest that there must be some limiting components that determine the rate at which a tip grows. These components must increase at the RCP causing a rise in the overall elongation rate as well as being involved in growth at the new end. A possible candidate here might be the numbers of vesicles or their microtubular 'rails', which are involved in cell wall growth at fungal tips (Byers & Goetsch, 1975). More of these components might be made available, followed by a redistribution of components already involved in old-end growth. Whatever the mechanism involved it is able to undergo periodic increases in the limiting component in the absence of progress through the cell cycle. Two processes must be playing roles in this system. One closely associated with nuclear behaviour, which can activate new-end growth; and another one independent of the nucleus, which is limiting overall cell elongation. A careful microscopic study of cells undergoing NETO and the rate change should illuminate these possibilities.

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J. M. Mitchison and P. Nurse


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