PROTEIN SYNTHESIS AND ITS RELATION TO THE DNA-DIVISION CYCLE IN THE FISSION YEAST SCHIZOSACCHAROMYCES POMBE

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SUMMARY
The rate of protein synthesis has been measured with pulse labels of [3H]tryptophan in synchronous and asynchronous cultures of cdc mutants of Schizosaccharomyces pombe shifted up to the restrictive temperature. The cell cycle related fluctuations in rate that occur in normal synchronous cultures vanish when nuclear division is blocked in synchronous cultures of cdc2 and cdc10. But they persist in cdc11 where nuclear division continues and cleavage is stopped. We conclude that nuclear division affects the rate of synthesis and that this effect is inhibitory and probably persists for the last 40% of the cycle. When nuclear division has been blocked, the rate of synthesis continues to increase until a plateau is reached where the rate remains constant. Three size mutants of cdc2 reach the plateau at the same average protein content per cell although their initial protein contents vary over a threefold range. Comparison of these results with those from cdc10 leads to the tentative conclusion that the plateau starts when the cells reach a critical protein/DNA ratio.

INTRODUCTION
We have shown that the rate of protein synthesis (as judged by tryptophan incorporation) does not increase exponentially through the cell cycle of the fission yeast Schizosaccharomyces pombe (Creanor & Mitchison, 1982). Instead, there is a sharp increase in rate at an 'acceleration point', which is at about 0.9 of the cycle. This periodic event in macromolecular synthesis does not appear to be associated with DNA synthesis since its location in the cycle remains unchanged in wee mutants where the S period is moved relative to wild-type cells. The acceleration point, however, might be associated with nuclear division or cell division, the other two events of the DNA-division cycle. To investigate this question, we have followed the rate of synthesis in asynchronous and synchronous cultures of cdc mutants that have been blocked in their traverse of the cell cycle by shifting to the restrictive temperature. We conclude that the acceleration point is linked to nuclear division. We have also found that the rate of synthesis in the blocked mutants continues to increase until it reaches a plateau. In the case of cdc2, the use of different alleles and a double mutant shows that the plateau starts at a constant average protein content per cell irrespective of the size before the block. cdc10 reaches the plateau with a lower protein content but also with a lower DNA content, and it may be that the attainment of a critical protein/DNA ratio is responsible for the beginning of the plateau.
MATERIALS AND METHODS

Organisms
The wild-type strain 972h~ was originally obtained from Professor U. Leupold, Bern. The mutants cdc2.33, cdc10.129 and cdc11.123 are temperature-sensitive conditionals that form colonies at 25°C but not at 35°C (Nurse, Thuriaux & Nasmyth, 1976). The cdc2+ function is required both for mitosis and for the initiation of DNA synthesis (Nurse & Bisset, 1981). cdc2.M35 is an allele that has a larger size at division at 25°C than cdc2.33 (Nurse & Thuriaux, 1980). The cdc10+ function is required for the initiation of DNA synthesis. cdc11.123 is an 'early cell plate' mutant and shows nuclear division without septum formation at the restrictive temperature. The double mutant cdc2.33 wee1.6 was constructed by the appropriate crosses and tetrad analysis (Benitez, Nurse & Mitchison, 1980).

Methods
All the methods have been described (Creanor & Mitchison, 1982) except for Giemsa staining of nuclei, which is given by Nurse et al. (1976), and measurements of DNA by the diphenylamine method (Bostock, 1970). The cultures were grown in a minimal medium EMM3 (Creanor & Mitchison, 1982). This was supplemented in some cases with small amounts of tryptophan and yeast extract, which reduce rotor perturbations.

RESULTS
The experiments all followed a common plan in which an exponential phase culture growing at the permissive temperature (25–30°C) was shifted to the restrictive temperature (36.5–37°C). The rate of incorporation of tryptophan was then measured using successive 10- to 12-min pulses of labelled tryptophan over a period of up to 7 h. Cell numbers were also measured as a control for the efficiency of the cell cycle block in the cdc mutants. Some experiments were done with normal asynchronous cultures, but in others 'synchronous' cultures were made by size selection in an elutriator rotor just before the temperature shift (Creanor & Mitchison, 1979). Although these cultures start with small cells and would normally show several synchronous divisions, they do not do so with the cdc mutants at the high temperature. In some cases, 'asynchronous control' cultures were made by passing cells through the rotor without size selection. These act as controls for possible perturbing effects of the rotor.

Wild-type 972h~
Wild-type cells (Fig. 1A) showed an exponential rise in the rate of incorporation over most of the period of the experiment with a doubling time of 130 min. For about the first 90 min, however, the rate rose somewhat faster. This initial period of rapid rate increase occurred in nearly all the cultures and is more marked in some of the mutant experiments. It is presumably a perturbation caused by the temperature shift. Cell numbers (Fig. 1B) also showed a perturbation for the first 90 min and then settled down to an exponential increase with a doubling time of 150 min, somewhat greater than the doubling time of the incorporation rate.

Mutant cdc2.33
Asynchronous cultures of cdc mutants shifted to the restrictive temperature show
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Fig. 1. Rate of tryptophan incorporation in asynchronous cultures of strain 972 (wild type) and mutant cdc2.33. The cultures were grown in EMM3 + 10 μg/ml tryptophan and were shifted from 28 °C to 36.5 °C at time zero. Curve A, 972, samples (0.25 ml) labelled for 10 min with 307 kBq [3H]tryptophan. Each point is the mean of two samples; 1 arbitrary unit (a.u.) = 234 c.p.m. Curve B, cell numbers in A; 1 a.u. = 0.33 x 10^6 cells/ml. Curve C, cdc2.33, samples as in A but with 263 kBq; 1 a.u. = 1420 c.p.m. Curve D, cell numbers in C; 1 a.u. = 2.67 x 10^6 cells/ml.

an initial rise in cell numbers as cells past the transition point finish the cycle and divide (Nurse et al. 1976). Thereafter there is little or no increase in cell number though growth continues and the cells become oversize. Fig. 1c and D shows incorporation rate and cell number for cdc2.33. The incorporation rate had the same initial rapid rise as in wild-type cells but it was more marked. After the first 100 min, the rate followed a slower exponential rise (doubling time of 160 min) for a further 140 min. At 4 h the rate reached a plateau in which there was little or no increase.

Although the events of the DNA-division cycle are blocked in cdc mutants at the restrictive temperature, some other properties of the cell, e.g. enzyme potential (Benitez et al. 1980), continue to show periodic cell cycle changes. We tested for this by examining incorporation rate in a synchronous culture (Fig. 2a). This culture did not, of course, show synchronous division at the restrictive temperature but a portion
of it transferred to the permissive temperature (30 °C) gave good synchrony with a cell plate (septal) index peak of 29% at the first division (data not shown). The incorporation pattern was very similar to that of the asynchronous culture in Fig. 1c. It should be compared to that in the synchronous wild-type culture in Fig. 2c (reproduced from Creanor & Mitchison, 1982). The mutant did not show the periodic acceleration points and the curved pattern between them that are characteristic of the normal cycle. There was, however, only about a single cycle time of exponential rate increase in the mutant before the terminal plateau and a more rigorous test for the absence of periodicity was done with the double mutant below.

Fig. 2. Rate of tryptophan incorporation in synchronous cultures of mutant cdc2.33 and of strain 972 (wild type). Curve A, cdc2.33 grown at 30 °C in EMM3 +10 μg/ml tryptophan + 0.025 % yeast extract, selected to produce a synchronous culture at time zero and shifted 10 min later to 36.5 °C. Samples (0.25 ml) labelled for 12 min with 370 kBq [3H]tryptophan. Each point is the mean of two samples. 10 arbitrary units (a.u.) = 801 c.p.m. Curve B, cell numbers in A; 10 a.u. = 0.85 x 10^6 cells/ml. Curve C, 972 grown at 30 °C in EMM3 +10 μg/ml tryptophan. Samples as in A but with 307 kBq; 1 a.u. = 1130 c.p.m. The arrows mark acceleration points. Curve D, cell numbers in C; 1 a.u. = 0.85 x 10^6 cells/ml. C and D show the same data as in Fig. 3A and B of Creanor & Mitchison (1982).
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Table 1. Average cellular protein and DNA content in temperature-shift experiments with asynchronous cultures

<table>
<thead>
<tr>
<th>Strain</th>
<th>Time from shift up (m)</th>
<th>Protein at time in col. (2) (pg/cell)</th>
<th>Time to start of rate plateau (m)</th>
<th>Protein at time (4) (pg/cell)</th>
<th>DNA at time (4) (pg/cell)</th>
<th>Protein/DNA at time (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>972h− (wild type)</td>
<td>120</td>
<td>16†</td>
<td>—</td>
<td>—</td>
<td>32†</td>
<td>0.50†</td>
</tr>
<tr>
<td>cdc2.33</td>
<td>20</td>
<td>16</td>
<td>240</td>
<td>40</td>
<td>32</td>
<td>1.25</td>
</tr>
<tr>
<td>cdc2.33.M35</td>
<td>20</td>
<td>26</td>
<td>170</td>
<td>41</td>
<td>29.5</td>
<td>1.39</td>
</tr>
<tr>
<td>cdc2.33 wee1.6</td>
<td>0</td>
<td>7.9</td>
<td>420</td>
<td>40</td>
<td>29</td>
<td>1.38</td>
</tr>
<tr>
<td>cdc10.129</td>
<td>120</td>
<td>14</td>
<td>360</td>
<td>29</td>
<td>21</td>
<td>1.38</td>
</tr>
</tbody>
</table>

*These are for unblocked cells.

Mutant cdc2.33

This mutant was used in order to test the effects of cell size on incorporation. It is an allele of cdc2, which is about 60% larger than wild-type or cdc2.33 (Table 1). The incorporation pattern (Fig. 3A) showed the same features as cdc2.33, but the plateau came earlier at 170 min. In spite of the considerable difference in initial protein content, the protein content at the start of the plateau was very similar at 40–41 pg/cell (Table 1).

Mutant cdc2.33 wee1.6

This small double mutant gave a second test of the effects of size since it was half the protein content of cdc2.33 (Table 1). The pattern was similar to that shown by cdc2.33 but the exponential rise in rate went on for much longer and the plateau was not reached until nearly 7 h (Fig. 3A). However, as with the large mutant, the size (protein content) at the start of the plateau was the same as that in cdc2.33 (Table 1).

The longer period before the plateau gave the opportunity for a more rigorous test for periodicities in a synchronous culture. Fig. 3C shows the absence of periodicities in a synchronous culture and a pattern similar to that in the asynchronous culture in Fig. 3B. In a part of the culture kept at 28°C, the midpoint of the number rise in the first synchronous division was at 3 h (data not shown). If there had been a periodic change in incorporation rate in the blocked culture, it should have been apparent during the exponential section from 1 to 5 h.

Mutant cdc10.129

The functional product of cdc10+ is required for DNA synthesis and the mutant has an early transition point. As a result there is a round of division after a shift-up in temperature and a doubling in cell numbers before division finally ceases and the cells
Fig. 3. Rate of tryptophan incorporation in asynchronous cultures of mutants *cdc2.M35* and *cdc2.33 wee1.6*, and a synchronous culture of *cdc2.33 wee1.6*. Curve A, asynchronous cultures of *cdc2.M35* grown at 25 °C in EMM3 and shifted at time zero to 36-5 °C. Samples (0-25 ml) labelled for 12 min with 370 kBq of [3H]tryptophan. Each point is the mean of two samples; 1 arbitrary unit (a.u.) = 162 c.p.m. Curve B, asynchronous cultures of *cdc2.33 wee1.6* grown at 28 °C in EMM3 + 10 µg/ml tryptophan + 0.025 % yeast extract and shifted at time zero to 36-5 °C. Samples as in A but labelled with 308 kBq for 10 min; 1 a.u. = 528 c.p.m. Curve C, synchronous culture of *cdc2.33 wee1.6*. Growth conditions and temperature shift as in B, and samples as in A; 1 a.u. = 258 c.p.m. Curve D, cell numbers in A; 1 a.u. = 1.04 x 10⁶ cells/ml. Curve E, cell numbers in B; 1 a.u. = 1.88 x 10⁶ cells/ml. Curve F, cell numbers in C; 1 a.u. = 1.60 x 10⁶ cells/ml.

start to become oversize (Nurse et al. 1976). The terminal phenotype has an unreplicated 1C amount of DNA whereas *cdc2* has a 2C amount.

Fig. 4A shows the pattern of incorporation, after a shift-up, of an asynchronous culture. There were the same general features as before with an initial rapid rise and a final plateau. The plateau was not reached until 5½ h, though division continued until 4 h. The protein content per cell at the start of the plateau was distinctly less (73 %) than in *cdc2* (Table 1).

Synchronous cultures showed one synchronous division at the restrictive temperature (Fig. 4D). The incorporation curve (Fig. 4C) showed the same pattern as
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Fig. 4. Rate of tryptophan incorporation in asynchronous and synchronous cultures of mutant cdc10.129. Curve A, asynchronous culture grown at 28 °C in EMM3 and shifted at time zero to 36.5 °C. Samples (0.25 ml) labelled for 10 min with 370 kBq of [3H]tryptophan. Each point is the mean of two samples. 1 arbitrary unit (a.u.) = 171 c.p.m. Curve B, cell numbers in A; 1 a.u. = 0.28 × 10^6 cells/ml. Curve C, synchronous culture grown at 28 °C in EMM3 + 10 μg/ml tryptophan and 0.025% yeast extract. Temperature shift and samples as in A except the labelling was for 12 min with 246 kBq; 1 a.u. = 673 c.p.m. Curve D, cell numbers in A; 1 a.u. = 0.76 × 10^6 cells/ml.

wild-type cells (Fig. 2c) for this initial cell cycle. The rate started to decelerate after the first hour, reached a plateau and then increased again at an acceleration point near the end of the cycle. Thereafter the pattern changed to the steady rate of increase characteristic of cdc2, followed by a final plateau.

We attempted to expand the exponential section by using the double mutant cdc10.129 wee1.6, but the cells lost viability by the end of the 9–10 h needed for this experiment.

Mutant cdc11.123

This is an 'early cell plate' mutant in which nuclear division continues at the restrictive temperature but septation and cleavage are blocked. The cells grow into
long filaments containing up to 16 nuclei (Nurse et al. 1976). The results from two synchronous cultures are shown in Fig. 5A–D. Cell numbers did not increase (data not shown) but there were two synchronous bursts of nuclear division, which increased the average number of nuclei/cell from 1 to 4. The incorporation curves had periodic fluctuations at cell cycle timings. These were not as marked as the fluctuations in the wild-type cells in Fig. 2a, nor was there a doubling in rate over each cycle but the acceleration points bore approximately the same relation to nuclear division as in

![Diagram](image-url)

Fig. 5. Rate of tryptophan incorporation in synchronous and asynchronous control cultures of mutant cdcl. Curve A, synchronous culture grown at 28°C in EMM3 + 10 μg/ml tryptophan + 0.025% yeast extract and transferred to 36.5°C immediately after selection at time zero. Samples (0.25 ml) labelled for 12 min with 308 kBq [³H]tryptophan. Each point is the mean of two samples. Initial cell number = 1.42 × 10⁶ cells/ml. 1 arbitrary unit (a.u.) = 44 c.p.m. Curve B, as for A but transferred to 37°C and labelled with 370 kBq. Initial cell number = 0.85 × 10⁶ cells/ml; 1 a.u. = 85 c.p.m. Curve C, average nuclei/cell in A; 1 a.u. = 0.17. Curve D, average nuclei/cell in B; 1 a.u. = 0.29. Curve E asynchronous control culture grown at 28°C in EMM3 + 10 μg/ml tryptophan + 0.025% yeast extract and transferred to 37°C after removal from rotor at time zero. Sampling as in A but labelled with 370 kBq; 1 a.u. = 878 c.p.m. Curve F, cell numbers in E; 1 a.u. = 1.55 × 10⁶ cells/ml.
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wild-type cells. We conclude that nuclear division in this mutant has a modulating effect on incorporation, which is similar but not identical to that which occurs in the wild type.

Fig. 5E and F show the results from an asynchronous control culture. The incorporation curve did not have the periodic fluctuations of the synchronous cultures, which shows that these fluctuations were genuine cell cycle events and were not the product of perturbation in the rotor. The rate continued to increase for the 5.8 h of the experiment and there was no sign of a final plateau.

Protein/DNA ratio

Table 1 shows the average protein and DNA content per cell at the start of the plateau and also the protein/DNA ratio. The three cdc2 mutants reached almost the same protein content at the start of the plateau although they began with very different protein values at or near the shift-up (column 3). cdc10, however, had a markedly lower protein content at the start of the plateau. The DNA values in column 6 need some comment. The value of 32 fg/cell for cdc2.33 is higher than would be expected. In view of the DNA block in this mutant, there should be an appreciable number of diploid cells that would give a total DNA value per cell less than the wild-type value of 32 fg/cell. The reason for the high value is likely to be the presence of a proportion of diploid cells since the initial amount of DNA at shift-up was 34 fg/cell (not shown in Table 1). With the double mutant cdc2.33 wee1.6 and with cdc10, the values for DNA nearer the time of shift-up were lower than those in column 6 (data not shown). Presumably there was some leakage into DNA synthesis during the long periods before the plateau.

Column 7 of Table 1 shows that the protein/DNA ratios of three out of the four mutants were very similar at the start of the plateau and were about 2.8 times the value in wild-type cells. The ratio for cdc2.33 was 9% lower. This might have been due to diploids, which could have behaved in a quantitatively different way from normal haploid cells, but there may also have been variation in the DNA assay, which is not very accurate especially between experiments. Within the limited range of mutants studied, we conclude that the main factor that starts the rate plateau is the attainment of a critical protein/DNA ratio and not the initial cell size or the type of mutant block.

DISCUSSION

In an earlier paper (Creanor & Mitchison, 1982), we showed that there were periodic fluctuations in the rate of protein synthesis during the cell cycle and that they were not associated with the S period. In this paper, we investigate whether or not they are associated with the other two main events of the DNA-division cycle: nuclear division and cell division. The fluctuations were absent in blocked 'synchronous' cultures of the mitotic mutant cdc2.33 and in the double mutant cdc2.33 wee1.6 where there is a longer period in which they might have been observed. In the case of the DNA mutant cdc10.129, a typical fluctuation occurred at the residual division after the temperature shift but there were then no further fluctuations. Continuing
fluctuations did, however, take place in the mutant cdc11.123 where septation and cleavage are blocked but nuclear division carries on. We conclude, therefore, that the fluctuations show temporal association with nuclear division but not with cell division.

In the absence of nuclear division, the rate of synthesis increases smoothly and exponentially between the two limits set by the end of the initial rapid rate of increase and the start of the rate plateau. The effect of nuclear division is inhibitory in the sense that it slows down and even stops the rate increase until the acceleration point at 0.9 of the cycle, well after mitosis at 0.75 of the cycle. We do not know why this happens. One explanation would be that RNA transcription stops during mitosis, as it does in most higher cells, and this affects the rate of protein synthesis. We have, however, presented evidence earlier (Creanor & Mitchison, 1982) that this does not happen in S. pombe. There are two other points that can be made. The first is that the inhibitory effect may last for an appreciable part of the cycle. Model fitting suggests that the rate of synthesis in single cells increases for the first 60% of the cycle and then remains constant for the remaining 40% (Creanor & Mitchison, 1982). If so, the start of the inhibitory effect is an early event in the preparations for mitosis. The second point is that the effect is not a late event triggered by the previous mitosis. If it were, it would have appeared once after the shift-up in cdc2 synchronous cultures and twice after the shift-up in cdc10.

cdc mutants continue to grow at the restrictive temperature and become oversize (Nurse et al. 1976). Indeed, it is on this criterion that most of the S. pombe mutants were selected. The detailed analysis in this paper shows that the rate of protein synthesis continues to increase for a period and then reaches a plateau of constant rate. The three cdc2 mutants reach this plateau at a constant size (protein content) but at very different times after the block. They are all then uninucleate with a 2C DNA value and oversize. cdc1 also grows oversize but it does not show a rate plateau within the period of our experiments. On the other hand, it is multinucleate and keeps the normal nucleocytoplasmic ratio. cdc10 behaves like cdc2 but has a 1C DNA value and reaches the plateau with a lower protein content. Although cdc10 has a lower protein content than the cdc2 mutants, it does have the same protein/DNA ratio at the start of the plateau. From this limited information, it appears that attainment of a critical value of this ratio may initiate the plateau.

Two similar studies have been done with blocked cdc mutants in S. pombe. The first was by Elliott (1983a,b) on the rate of ribosomal RNA synthesis, using 13- to 16-min pulses of [3H]uridine. There were substantial similarities between his results and those on protein synthesis described in this paper. RNA synthesis showed the same fluctuations in rate during the normal cell cycle, though the acceleration point was marginally earlier. These fluctuations vanished in blocked synchronous cultures of wee1.6 cdc2.33 and wee2.1 cdc10.129 but persisted in cdc11.123. He concluded, as we do, that the fluctuations are associated with nuclear division. The rate of RNA synthesis also showed the same plateau in a blocked culture. He called this plateau the 'transcription maximum' and, by analogy, we could call the protein plateau the 'translation maximum'. There was, however, a difference in the much shorter time to
the start of the RNA plateau and consequently the smaller size of the cells at that point. In *cdc2.33*, for example, the RNA plateau starts at a mean protein content per cell of 17 pg compared to 40 pg for the protein plateau. In addition, the results from a much wider range of mutants do not support the hypothesis that the RNA plateau starts at a constant protein/DNA ratio. For instance, with the protein plateau, this ratio is the same in *cdc2.33 weel.6* and in *cdc10* while with the RNA plateau the ratio for the double mutant is 60% of that for *cdc10*. Further tests of the protein plateau in other mutants would be needed to establish whether or not the maximal rates of RNA and protein synthesis are controlled by different mechanisms.

The second similar study with blocked mutants in *S. pombe* was on arginase and sucrase potential by Benitez et al. (1980). Enzyme potential is the maximum rate of increase in activity in samples taken from a culture and induced for that enzyme. In synchronous cultures of wild-type cells, the potential for these two enzymes increased in a sharp step once per cycle. In asynchronous cultures of blocked mutants, the potential patterns were similar to those for the rate of protein synthesis, with a rise followed by a plateau. The plateau occurred at a cell size much nearer to that for protein rate than for RNA rate, though this size was always greater for sucrase than arginase. In addition, the protein/DNA ratio was the same at the start of the plateau in seven mutants. In spite of these similarities, there was one conspicuous difference in that the cell cycle steps continued in blocked synchronous cultures. Therefore, the steps are not closely linked to the DNA-division cycle.

We should point out that we have assumed that the rate of tryptophan incorporation is a measure of the rate of protein synthesis. We have taken pains to justify this assumption in the normal cell cycle (Creanor & Mitchison, 1982) but we have not done so in this work with the *cdc* mutants. It remains possible, therefore, that some of the patterns are affected by changing pool sizes.

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REFERENCES


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