The kinetics of H1 histone kinase activation during the cell cycle of wild-type and wee mutants of the fission yeast *Schizosaccharomyces pombe*

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SUMMARY

H1 histone kinase activity has been followed in selection-synchronised cultures of fission yeast wild-type and wee mutant cells, and in induction-synchronised cells of the mutant cdc2-33. The main conclusions are: (1) in all three cases, the peak of activity is near mitosis. (2) The rise in activity is relatively slow starting in wild type at 0.4 of the cycle before mitosis. It is proposed that the beginning of the rise is the first identified event in the mitotic control. (3) The rise is twice as fast in wee and starts nearer to mitosis. (4) In all cases the beginning of the rise is in G2. (5) The fall in activity is also slow, lasting for 0.25 of the cycle, in wild type. Exit from mitosis happens well before activity has fallen to baseline. (6) In a range of size mutants, activity is roughly proportional to cell size. It is suggested that the kinase may have a cytoplasmic function. (7) Estimates have been made of the timing of mitosis in the mutants. In wee, mitosis is 0.14 of the cycle earlier than in wild type because the cell sizes have a longer septated period at the end of the cycle. (8) A novel method has been developed for eliminating the effects of the partial asynchrony in synchronous cultures, without which the kinetic analysis would have been inaccurate.

Key words: cdc mutant, cell cycle, fission yeast, H1 histone kinase, mitosis, mitotic control, p34^cdc2, *Schizosaccharomyces pombe*, size mutant, synchronous culture, wee mutant

INTRODUCTION

The gene product p34^cdc2 is a protein kinase that occupies a central position in the mitotic control of a wide range of eukaryotic cells (Nurse, 1990). When complexed with B type cyclin, it phosphorylates H1 histone in vitro and it is widely believed that this kinase activity triggers the onset of mitosis. We have therefore made a careful study of the kinetics of p34^cdc2 activation in fission yeast paying particular attention to the rise in activity that precedes mitosis.

Moreno et al. (1989) showed that H1 histone kinase activity in synchronous cultures of the fission yeast mutant cdc2-33 at the permissive temperature (25°C) rose to a peak near mitosis. We have repeated and confirmed these experiments in wild-type cells and we have also extended them to wee mutants and induction synchrony with cdc2-33. Our experiments have also involved estimates of the timing of mitosis in the mutants and the development of a new method of compensating for the partial asynchrony of synchronous cultures, which is particularly important when comparing wild-type cells with wee mutants.

MATERIALS AND METHODS

Strains

The wild-type (WT) strain 972h− of *Schizosaccharomyces pombe* was originally obtained from Professor U. Leopold, Bern. The mutants cdc2-33 and cdc25 are temperature-sensitive conditionals, which form colonies at 25°C but not at 35°C. Their functions are required for mitosis (Nurse et al., 1976; Fantes, 1979). In addition, the cdc2-33 function is required for an event in G1 (Nurse and Bisset, 1981; Novak and Mitchison, 1989). cdc2-M35r20 is a revertant that grows at 25°C and 35°C and is larger at division than WT at both temperatures. The alleles cdc2-22 and cdc2-M51 are larger than WT at 25°C. cdc2-lw, cdc2-3w and wee-6 are smaller than WT at 25°C and 35°C, whereas wee-50 is temperature-sensitive and is small at 35°C but larger at 25°C (Thuriaux et al., 1978; Fantes, 1981). We are grateful to Dr P. Nurse and Dr P. Fantes for supplying some of these strains.

Growth and synchronous cultures

Most cultures were grown at 25°C or 35°C in a shaking water bath in EMM3, a minimal medium with phthalate buffer (Creanor and Mitchison, 1982). Selection-synchronised cultures were prepared by elutriation (Creanor and Mitchison, 1979). The method used for induction synchrony with cdc2-33 is described in Results.

H1 histone kinase assay

The activity of H1 histone kinase (H1K) was measured in crude cell extracts by the following method. Samples of 25×10^6 cells were collected on membrane filters, washed twice with phosphate buffered saline at pH 7.3 (PBS, Oxoid) and then either broken at once or frozen at −20°C. For breakage, a sample was resuspended in 120 μl of ice-cold kinase assay buffer (KAB, see below) and 260 mg of 40-mesh glass beads added. The cells were broken by shaking on a Whirlimixer with a head modified to hold Eppendorf tubes horizontally.
Fresh cells needed 2 shaking periods of 1.5 minutes and frozen cells needed 3 periods. The supernatant was removed and centrifuged for 10 minutes in a Hawksley micro-haematocrit centrifuge to pellet any debris; 5 μl of this supernatant were then assayed for protein using the Pierce BCA protein assay. Each sample was then diluted with KAB buffer to give a protein concentration of 0.4 μg/μl. These samples can be preserved at -20°C, provided p-nitrophenyl phosphate is absent.

For the kinase assay, 10 μl of the diluted sample were added to 10 μl of substrate containing 1 mg/ml H1 histone (Boehringer), 200 μM ATP in KAB buffer, 100 μCi/ml [γ32P]ATP and 15 mM p-nitrophenyl phosphate. This mixture was incubated for 10 minutes, usually at 35°C and the reaction stopped by adding 20 μl of sample buffer and boiling for 3 minutes.

The samples were electrophoresed in linear 10% or 11% polyacrylamide gels. The gels were dried and autoradiographs made on Kodak X-omat film. The H1 histone bands on the autoradiographs were measured with a LKB densitometer. The bands were in the linear part of the exposure/density curve for the film. The ordinates in the Figures are given in arbitrary units of H1 kinase (per unit protein).

The KAB buffer (pH 7.2) contained 60 mM Na₂β-glycerophosphate, 25 mM MOPS, 15 mM MgCl₂, 10 mM EDTA, 5 mM EGTA, 0.2% Tween-20. To this was added, just before breaking, 1 mM Na orthovanadate, 1 mM dithiothreitol, 20 μg/ml leupeptin and 40 μg/ml aprotinin.

Other methods
Cell numbers were measured with a Coulter Counter (Industrial model D) with a 100 μm aperture. Counts of septated cells were made with dark ground illumination under a ×20 objective. Nuclei were stained with DAPI using the method of Moreno et al. (1991) and observed with a ×40 objective and fluorescence. Protein was measured by the Pierce BCA assay.

RESULTS

H1 histone kinase activity in synchronous cultures of wild-type cells
Fig. 1A,B shows the H1 histone kinase (H1K) activity and cell numbers in a selection-synchronised culture of wild-type (WT) cells grown in minimal medium EMM3 at 35°C. There are peaks in H1K activity before division at about the time of mitosis that are separated by a plateau in interphase where there is still appreciable activity. This pattern is very similar to that found by Moreno et al. (1989) though the plateau is somewhat longer in their results. They were using the mutant cdc2-L7 at the permissive temperature of 25°C.

Because of the variability of the H1K assay, we repeated this experiment a further six times in order to get an accurate average curve. In each experiment, the timing was determined for five points: the start and finish of the plateau, the half-rise point to the peak, the peak, and the half-fall point after the peak. The average timings are shown in Fig. 2 together with standard error bars. These timings are in fractions of a cycle of 134 minutes (mean value for WT). The H1K activity was set at 1.0 for the plateau and the peak was at 3.97 since the average factor of peak activity/plateau activity was ×3.97 (n=6, s.e.m.=0.89). All synchronous cultures show imperfect synchrony and for accurate determinations of timing it is important to eliminate its effects. We have therefore developed a novel way of doing this so that is described in detail in the Appendix. In summary, it consists of making a simple model of what is happening in the average single cell and then correcting this for the asynchrony of the culture. The corrected model curve is compared to the experimental curve and, if the fit is good, the model curve shows what is happening at the single cell level.

Both the model curve and the corrected model curve are shown in Fig. 2 and it can be seen that the corrected curve gives a reasonably good fit to the experimental data. With these WT cultures the effect of correction is relatively small with a slight drop in the peak and some increase in the tails. This is because the synchrony is good and the rate of division curve is relatively narrow (see Fig. 8 in the Appendix) so the main shape of the corrected curve comes from the model where the H1K activity starts to rise at 0.3 of the cycle, peaks at 0.7, and falls back to baseline at 1.0. This is slightly asymmetric with the fall steeper than the rise. Narrower or broader models do not fit as well to the experimental curve. Indeed, the model curve is compared to the experimental curve and, if the fit is good, the model curve shows what is happening in the average single cell and then correcting this for the asynchrony of the culture. The corrected model curve is compared to the experimental curve and, if the fit is good, the model curve shows what is happening at the single cell level.

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Fig. 1. (A) H1 histone kinase activity (arbitrary units) in a selection-synchronised culture of WT cells at 35°C. (B) Cell numbers in the same experiment.

![Fig. 2. H1 histone kinase activity (arbitrary units) in WT cells against stage of the cycle (134 minutes). Mean experimental results (+ s.e.m.) (△), model curve (+) and corrected model curve (○) (see text). M, mitosis; S, mid-point of S period.](image-url)
fit the experimental curve after correction (Fig. 10). The reasons for timing mitosis at 0.69 of the cycle are given later. It is very near the activity peak.

**H1K activity in synchronous cultures of wee1 mutants**

Fig. 3A,B shows the H1K activity in a selection-synchronised culture of *wee1-50* cells at 35°C. The pattern is similar to that in WT cells but the peaks of activity are lower, and earlier with respect to the mid-point of the cell number steps. This experiment was repeated a further four times. Two synchronous cultures were also made with *wee1-6*. The results were very similar to those with *wee1-50* and have been added in to give the average experimental curve from seven cultures in Fig. 4 prepared in the same way as in Fig. 2 for WT except that the cycle time was taken as 148 minutes.

![Fig. 3.](image)

**Fig. 3.** (A) H1 histone kinase activity (arbitrary units) in a selection-synchronised culture of *wee1-50* cells at 35°C. (B) Cell numbers in the same experiment.

![Fig. 4.](image)

**Fig. 4.** H1 histone kinase activity (arbitrary units) in *wee1-50* and *wee1-6* cells against stage of the cycle (148 minutes). Mean experimental results (+ s.e.m.) (---), model curve (+) and corrected model curve (○) (see text). M, mitosis; S, mid-point of S period.

Fig. 4 also shows a model that, after correction, gives a good fit to the experimental data. The correction process has a much more marked effect than in WT because of the poorer synchrony and the broader rate of division curve (Fig. 8 in the Appendix). The model is narrower than that for WT, starting to rise at 0.37 of the cycle to a peak timing at 0.57 and then falling back to baseline at 0.77. Note, however, that the peak activity value of the model at 4.2 is about the same as that for the WT. Mitosis is earlier in *wee1* than in WT (see below) and is very near to the activity peak.

**H1K activity in induction synchrony with cdc2-33**

Synchrony can be induced in *cdc2-33* by blocking division for a period at the restrictive temperature and then releasing the block by shifting down to the permissive temperature (Creanor and Mitchison, 1989; Novak and Mitchison, 1990). The cells at the time of shift-down are larger than WT cells and they then divide with shortened cell cycles.

Fig. 5A,B shows the H1K activity in a synchronous culture prepared by growing *cdc2-33* at 28°C, shifting up to 35°C for 4 hours and then shifting back to 28°C. There are peaks in activity some time before the number steps. A repeat experiment gave almost identical results and the two experiments were meaned to give the average experimental curve in Fig. 6 with the abscissa as fractions of a cycle of 100 minutes. A problem with these experiments is that the block is imposed by denaturing the gene product *cdc2*, which is an integral part of H1K activity and it is uncertain what is happening during the recovery before the first division. We have therefore concentrated on the activity patterns before and after the second division and it is these that have been used in Fig. 6.

As before, Fig. 6 shows a corrected model curve that is in good agreement with the experimental data. The rate of division curve for the second division is markedly asymmetric.

![Fig. 5.](image)

**Fig. 5.** (A) H1 histone kinase activity (arbitrary units) in an induction-synchronised culture of *cdc2-33*. Culture grown at 28°C, shifted up to 35°C for 4 hours, and then shifted down to 28°C at time zero. (B) Cell numbers in the same experiment.
with a long late tail (see Fig. 8 in the Appendix). This may be due to the minority of cells that were blocked at the first transition point of cdc2-33 and therefore divided later than the majority of cells blocked at the second transition point. This is partially allowed for in the correction process but there is still a slightly imperfect fit in the tail. The model is very similar in shape to that used for the wee cells in Fig. 4, starting to rise at 0.2 of the cycle before the peak and falling to the baseline at 0.2 of the cycle after the peak. Mitosis is timed at 0.35 of the cycle, which is slightly after the peak in H1K activity, but there are some uncertainties about the exact timing, which are discussed later.

H1K activity in asynchronous cultures of size mutants

H1K activity and protein content were measured in asynchronous cultures of a range of size mutants and the results are presented in Table 1. The activity in cdc2-3w was unusually low but, apart from this mutant, the activity per unit of soluble protein showed comparatively little variation (a range of ±1.38) whereas the activity per cell or per nucleus varied considerably (a range of ±9.2). Large cells therefore have greater activity and the activity is roughly proportional to cell size. Note that for equal values of the peak activity in wee and WT the average activity in asynchronous cultures will be lower in wee because the rise and fall of activity occupies less of the cycle.

H1 histone kinase in cdc2-33 assayed at different temperatures

p34cdk2 can be purified from crude extracts by immunoprecipitation or by adsorption on p34cdk2 bound to Sepharose beads. We have not used such methods because they increase the variability of the H1K activity assay. We have, however, tested whether kinases other than p34cdk2 are phosphorylating H1 histone by making a selection-synchronised culture of cdc2-33 at 25°C and then assaying samples at 25°C and 40°C. The mean of 4 samples round the activity peak was reduced in the 40°C assay to 14% of that in the 25°C assay. We conclude that 86% of the activity peak is due to the thermolabile p34cdk2 gene product in this mutant. It is uncertain whether the 14% is a residual activity of the p34cdk2 complex or the activity of another kinase. The absence of a marked cell cycle pattern in this 14% suggests the latter alternative. Similar experiments by Moreno et al. (1989) also showed no marked cell cycle pattern in residual activity when assayed at 37°C in cdc2-L7.

Timing of mitosis and the length of G2

The only easy way of timing mitosis is to observe it by phase-contrast microscopy in cells growing in a high refractive index medium. With this method, Hagan (1988) found the time of nuclear separation (mitotic anaphase) as 0.69 of the cycle in WT 972, and we use this value. It is more reliable than the timing of 0.76 in Mitchison (1989) which came from a different strain.

If mitosis in wee mutants happened as late in the cycle as in WT cells, there would a serious discrepancy between the peak of H1K activity and mitosis. It is in fact earlier. Nasmyth et al. (1979) found the septation index in asynchronous cultures to be higher in wee1-1 and cdc2-1w than in WT. We have confirmed and extended their results (Table 2). The length of the septated period is about 50% greater in most cells with wee phenotypes than in WT, though the reason for this is unknown. The effect is to position mitosis earlier than in WT. An interesting but mysterious result is that cdc2-3w is like WT rather than in Mitchison (1989) which came from a different strain.

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### Table 1. Histone kinase activity and protein content in size mutants

<table>
<thead>
<tr>
<th>Strain</th>
<th>Total protein pg/cell</th>
<th>Soluble protein pg/cell</th>
<th>Activity/soluble protein</th>
<th>Activity/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>cdc2-1w</td>
<td>9.0</td>
<td>6.0</td>
<td>1.26</td>
<td>0.83</td>
</tr>
<tr>
<td>cdc2-3w</td>
<td>9.7</td>
<td>7.3</td>
<td>0.83</td>
<td>0.52</td>
</tr>
<tr>
<td>wee1-6</td>
<td>10.5</td>
<td>6.5</td>
<td>1.13</td>
<td>0.73</td>
</tr>
<tr>
<td>wee1.30</td>
<td>11.6</td>
<td>7.7</td>
<td>1.14</td>
<td>0.88</td>
</tr>
<tr>
<td>WT</td>
<td>15.7</td>
<td>9.7</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>cdc2-M35r20</td>
<td>13.2</td>
<td>1.38</td>
<td>1.0</td>
<td>0.49</td>
</tr>
<tr>
<td>cdc2-22</td>
<td>22.7</td>
<td>14.8</td>
<td>1.30</td>
<td>1.97</td>
</tr>
<tr>
<td>cdc2-25-M51</td>
<td>97.3</td>
<td>75.3</td>
<td>1.18</td>
<td>6.75</td>
</tr>
</tbody>
</table>

Mean values in asynchronous cultures in EMM3 at 25°C (except for wee1-50 at 35°C). Activities scaled to 1.0 for WT.

### Table 2. Septation index and length of septated period

<table>
<thead>
<tr>
<th>Strain</th>
<th>Septation index (%)</th>
<th>Length of septated period (fraction of cycle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>9.1</td>
<td>0.13</td>
</tr>
<tr>
<td>wee1-6</td>
<td>13.9</td>
<td>0.19</td>
</tr>
<tr>
<td>wee1-50</td>
<td>15.1</td>
<td>0.20</td>
</tr>
<tr>
<td>wee1-6 cdc2-33</td>
<td>13.0</td>
<td>0.18</td>
</tr>
<tr>
<td>cdc2-1w</td>
<td>14.8</td>
<td>0.20</td>
</tr>
<tr>
<td>cdc2-3w</td>
<td>9.1</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Septation index (% cells with septa) from 1000 cells at 35°C in EMM3, avoiding diploids when present. Length of septated period from the formula in Mitchison (1971). The true septation indices may be higher because the earliest stages of septum formation are invisible with low power microscopy.
than \( cdc2-1w \). That this is not due to a size difference can be seen from Table 1.

Although it would be difficult to detect the start of mitosis or the start of septation in synchronous cultures, it is possible to find the timing of the peak of binucleate cells without septa (BN), which is the nearest event to mitosis that is easily measurable under these conditions. Such peaks are shown in Fig. 7 for WT, \( wee1-50 \), and induction synchrony with \( cdc2-33 \).

From repeat experiments, the mean time of the BN peak in WT was at 0.84 of the cycle, which is 0.15 after mitosis at 0.69. The mean time of the BN peak in \( wee \) was 0.70. If it is assumed that there is the same difference between mitosis and the BN peak in \( wee1 \) as in WT, then mitosis in \( wee1 \) is at 0.70-0.15=0.55.

The situation with induction synchrony is more complicated. For a cycle time of 100 minutes, the BN peak is at 0.64 of the cycle. With the same assumption above, mitosis would be 0.15 of the cycle earlier - at 0.49 and well away from the H1K activity peak. We believe this is misleading because, unlike selection synchrony, there are two different 'cycles' in induction synchrony. One is the short cycle from division to division. The other is the much longer doubling time for events of growth such as optical density and \( CO_2 \) production, which is close to the cycle time of 193 minutes for this mutant in normal growth at 28°C (Creanor and Mitchison, 1989; Novak and Mitchison, 1990). We assume that the spacing between the cycle events of mitosis, the BN peak and cell division is dependent on growth rate. In this case mitosis is at 0.35 of the cycle of 100 minutes, close to the H1K activity peak. Obviously, there is some uncertainty here because of the assumption about spacing.

It is of interest to know when in G2 the H1K activity in the models starts to rise. Data on this are presented in Table 3. They show that the rise starts near the beginning of G2 in \( wee1 \) cells and about half way through the G2 periods in WT and in induction synchrony. The precision of these figures is not high both because it is difficult to determine the exact time of the start in the experimental curves and also because more complex models with extended tails might also give a reasonable fit to the experimental data.

**DISCUSSION**

Our results on WT cells confirm those of Moreno et al. (1989) on the mutant \( cdc2-L7 \) at 25°C in showing that the peak of H1K activity is very near to the start of mitotic anaphase. Our results also show that the same is true both for \( wee1 \) mutants and for induction synchrony using block and release of the \( cdc2-33 \) mutant, provided the altered times of mitosis in these situations are taken into account. They are in accord with the widely held view that the approach to the peak of H1K activity is the immediate stimulus for mitosis (e.g. see Nurse, 1990).

The most important conclusions come from the kinetics of the rise and fall in activity. An interesting finding is the rela-

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**Table 3. G2 period and start of rise of H1K activity**

<table>
<thead>
<tr>
<th></th>
<th>Mid-point of S phase</th>
<th>Length of G2</th>
<th>Start of rise in H1K activity model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fraction of cycle</td>
<td>Minutes</td>
<td>Fraction of cycle</td>
</tr>
<tr>
<td>WT</td>
<td>0.0*</td>
<td>86</td>
<td>0.05 to 0.69=0.64</td>
</tr>
<tr>
<td>( wee1 )</td>
<td>0.29†</td>
<td>31</td>
<td>0.35 to 0.55=0.20</td>
</tr>
<tr>
<td>Induction</td>
<td>0.67‡</td>
<td>58</td>
<td>0.77 to 0.35=0.58</td>
</tr>
</tbody>
</table>

S period assumed to last for 0.1 of cycle in WT (Mitchison, 1989) and \( wee1 \), and 0.19 in induction (=0.1x193/100). G2 assumed to finish at start of nuclear separation.

†Nurse and Thuriaux (1977) for \( wee1-50 \) at 35°C.
‡Novak and Mitchison (1990) for second cycle.
tively slow rise in activity commencing about halfway through G₂. The long G₂ in WT means that this commencement is 0.4 of the cycle before mitosis. We suggest that this commencement is an important event of the mitotic control and that it is the earliest identified event. It in turn will be controlled by other elements, two of which are likely to be the inhibitory effect of the wee1+ kinase and the stimulating effect of the cdc25+ phosphatase (Nurse, 1990). Possibly the balance between them has to reach a critical ratio in G₂ before the H1K activity starts to rise, but the details of this balance are unknown. The kinase is missing in wee mutants and the commencement of the activity rise in the model is near the beginning of the shortened G₂. This in turn suggests another (checkpoint) element in the control, that the activity rise cannot begin before the end of the S period even though there is no inhibition by the wee kinase.

The question of the rate of rise of activity underlines the importance of the method we have used to allow for the difference in rates of division in synchronous cultures of WT cells and wee1 mutants. Most people looking only at the mean experimental curves for wee1 (Fig. 4) and for WT (Fig. 2) would conclude that the activity in wee1 started to rise in late G₁ and that thereafter the rate of rise was less than in WT. This conclusion would be false because it ignores the different rates of division. The model curves for the average single wee1 cell show that the rise starts very early in G₂ and then rises at twice the rate in WT cells. Note here that this applies to an average rate of rise. The models used have simple linear rises at a constant rate and some more complex models with increasing rates of rise (as in an autocatalytic process) might also fit the experimental curves after correction.

The rates of fall in activity in the models are also fairly slow. In WT cells, the fall lasts all the way through G₁, about 0.25 of the cycle. In wee1 the fall is quicker and reaches base-line about 30% of the way through the long G₁ of 0.7 of the cycle. In the same way that the entry into mitosis may occur on reaching a critical activity level before the peak, so also may the exit from mitosis be permitted when the activity has fallen to a critical level rather than to baseline.

There is excellent evidence (reviewed by Nurse and Fantes, 1981) that there is a size control that ensures that WT cells have to reach a threshold size before mitosis, though the molecular basis is unknown. If this size has to be reached before the mitotic controls begin to operate, our results indicate that this happens at least 0.4 of the cycle before mitosis, which is considerably earlier than was supposed.

Relatively slow rises in H1K activity have also been found in other cell cycles. Synchronous cultures of mouse FT210 cells (a temperature-sensitive cdc2 mutant) at the permissive temperature showed a slow rise in H1K activity after immunoprecipitation with antibodies against p34cdc2 and against human B1 cyclin (Hamaguchi et al., 1992). The rise started in G₁ but accelerated markedly in late S and G₂. It is possible that the slow initial rise might be due to the method of induction synchronisation. In Physarum, another growing system, there are two nuclear H1 histone kinases whose activities started rising at the beginning of G₂ (about 5 hours before mitosis) and peaked before mitosis (Bradbury et al., 1974; Hardie et al., 1976). Ducournou et al. (1990) found a much sharper rise in p34cdc2 kinase activity in Physarum with a peak at metaphase. Even so, their Fig. 1 shows the rise starting in late G₂ about 1 hour before mitosis. In extracts of Xenopus eggs, a non-growing system with very short cell cycles, Murray and Kirschner (1989) found that the rise in H1K activity occupied about half the cycle and there was no lower plateau in activity. In contrast, an assay for MPF (maturation promoting factor) showed a sharper rise and a distinct lower plateau.

Fluorescence microscopy has shown that the p34cdc2/p63cdc13 complex, believed to exhibit the H1K activity, is localised in the nucleus (e.g. Alfa et al., 1991). Our data on the size mutants in Table 1 show that the H1K activity increases per cell (or per nucleus) in large cells. This suggests that although the main target of the activity may be nuclear there are also cytoplasmic targets e.g. the cytoplasmic microtubules. Changes in activity per nucleus were also found by Murray and Kirschner (1989) in cycling extracts of Xenopus eggs. They found that the peak of H1K activity per unit protein was the same in two successive cycles. Since the number of nuclei will have increased between the cycles, the activity per nucleus will have decreased. Gerhart et al. (1984) found a similar result with MPF assays from intact embryos in the first two cycles.

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APPENDIX

A method of eliminating the effects of partial asynchrony in synchronous cultures

A few cellular systems, such as early embryos and Physarum polycephalum, have perfect or near perfect natural synchrony, but most synchronous cultures made by selection or induction show imperfect synchrony, which manifests itself in the appreciable time taken for the doubling in cell numbers at division and which increases in successive cycles (Mitchison, 1971). This needs to be allowed for in accurate studies of the timing of cell cycle events such as, for example, the rise and fall in H1 histone kinase activity.

We have developed a method of allowing for imperfect synchrony that is simpler than that given by D. A. Williams in the Appendix of Creanor and Mitchison (1982) and can be applied to any type of synchronous culture. In essence, this involves making a simple model of what is assumed to be the kinetics of kinase activity in the average single cell or in a perfect synchronous culture where all the cells divide at the same time. This model is then corrected and spread out by being weighted by the real rate of division at intervals during the period of division. The corrected curves for various models are then compared with the experimental data to see which one gives the best fit. This eliminates the effects of partial asynchrony and allows a valid comparison between synchronous cultures that have different rates of division (e.g. WT and wee cells).

Step by step details of the method are as follows, using data in this paper as examples.

1. Construct a curve for the rate of division against time as fractions of the cycle. For our data, the rate of division was
found from the difference between successive cell number counts (at 15 minute intervals) in a representative culture and (for convenience) normalised to a peak value of 1.0. These curves were very similar in repeat experiments on the same strain but differed between strains. Three such curves are shown in Fig. 8. The curve for wee1-50 is broader than that for WT and the peak was lower before normalisation. This reflects the longer time for the doubling of cell numbers at division in a wee synchronous culture. The curve for induction synchrony is markedly asymmetric (see Results for a possible explanation). In other systems, curves for mitotic index (percentage cells in metaphase) could be substituted.

(2) Choose a model curve for cell cycle event against time for the average single cell. We have used simple models with linear rises and falls, but more complex models could be used.

(3) Construct a table of rows and columns. Each column represents time and each row contains model data. For the WT model shown in Fig. 2, there were 12 columns at 0.1 of the cycle intervals from 0.4 to 1.5, and 5 rows. The central row contained the model curve for cells dividing in the middle of the number step with the maximum rate of division. It was positioned with the model peak in the column at 1.0 of the cycle and all the values multiplied by the height of the normalised rate of division curve at this stage of the cycle (1.0). The values ranged from a baseline of 1.0 to a peak of 4.2. The row above was for cells dividing at 0.9 of the cycle. The model was positioned with its peak in the column at 0.9 of the cycle and the values multiplied by the height of the rate of division curve at that time (0.61 from Fig. 8). The top row, for cells dividing at 0.8, had its peak at 0.8 and a multiplication factor of 0.11. A similar procedure was used for the cells dividing later than 1.0 of the cycle. These rows of model curves are shown graphically in Fig. 9.

(4) Sum each column to give the corrected model curve. With our data, the values were scaled to give a baseline value of 1.0, and the abscissa shifted by -0.3 of the cycle to position the peak at 0.7 (the experimental peak). Compare the corrected model curve with the experimental data. If the fit is unsatisfactory, go back to (2) above and try another model of different shape or height.

The shape of the corrected curves is affected both by the shape of the model curves and by the shape of the rate of division curve. An example of the sensitivity to the models is shown for WT in Fig. 10. Fig. 10A shows three model curves with baseline breadths of 0.4, 0.7 and 1.0 of the cycle. The differing peak values have been chosen so as to give the same peak value after correction. The corrected curves for these three models are shown in Fig. 10B together with the average experimental curve. It can be seen that model B with a baseline breadth of 0.7 of the cycle gives, after correction, a reasonably good fit to the experimental data and the other two models do not.

Fig. 8. Rate of division in synchronous cultures (scaled to 1.0 at peak) against stage of the cycle for WT (+), wee1-50 (∆), and induction synchrony of cdc2-33 (2nd cycle) (○).

Fig. 9. Family of model curves for WT before summing. Each curve is one of the rows in the correction table.

Fig. 10. H1 histone kinase activity against stage of the cycle.
(A) Three model curves with base widths of 0.4, 0.7 and 1.0 of the cycle. Model A, ∆ (0.5-0.9); model B, ○ (0.3-1.0); model C, + (0.1-1.1). (B) The same models after correction by the rate of division in WT cells, together with the mean experimental data for WT cells from Fig. 2 (○-○).
This method assumes that the variation in the timing of an event such as the start of the rise in kinase activity is the same as the variation in the time of cell division. Since the variation in division time is greater in the second synchronous division than in the first (an effect that is much less marked in wee cultures than in WT) the question arises as to which rate of division curve is used for weighting the models. In general, we have used the rate of division curve that was nearest in time to the events in the model. If the variation in events increases steadily through the cycle (an assumption) then the variation will be less for events before division and greater for events after division.

REFERENCES


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