EFFECTS OF TEMPERATURE AND NUTRITIONAL CONDITIONS ON THE MITOTIC CELL CYCLE OF SACCHAROMYCES CEREVISIAE

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SUMMARY

Yeast cells were cultivated at different growth rates in a chemostat by alterations in the flow of the limiting nutrient glucose and in batch cultures where variations in growth rate were achieved by alterations in the composition of nutrients. It was observed that the stage in the cycle at which S-phase was completed varied with growth rate. The faster the growth rate, the earlier the stage in the cycle in which completion of S-phase occurred. When stage in the cycle is converted into time before division it was observed that the time from completion of S-phase to cell division varied only slightly with growth rate except at extremely slow growth rates. Expansion of cell cycle transit time as the growth rate was slowed was achieved primarily by an expansion in time of the period from division to the completion of S-phase.

In contrast, when cells were grown at different rates by alterations in the temperature of cultivation, completion of S-phase occurred at approximately the same stage in the cell cycle at all growth rates.

INTRODUCTION

While extensive studies have been made in higher eukaryotes and especially mammalian cells on the effect of environmental conditions on the lengths of different phases of the cell cycle, relatively few studies have been made in lower eukaryotes (Mitchison, 1971; Prescott, 1976; Carter, 1977).

Yeast is a lower eukaryote in which the basic cell structure and mechanisms of macromolecular synthesis as well as the replication and segregation of chromosomes are similar to those of higher eukaryotes. The mitotic cell cycle of yeast like other eukaryotes is made up of 4 distinct phases: G1, S, G2 and M. Yeast cells can be grown at a variety of rates in a chemostat with glucose as the limiting nutrient. In addition, variations in generation time can be obtained in batch cultures using media of different nutrient composition. Growth rate can also be altered by changing the temperature at which the cells are growing.

In the present communication we have altered the growth rate by changes in medium composition while maintaining a constant temperature and by changes in temperature while maintaining a constant medium composition, and we have examined whether alterations in the cell cycle length produced by these conditions result in changes in the length of time from completion of DNA synthesis to cell division.
MATERIALS AND METHODS

*Saccharomyces cerevisiae* strain A364a ade 1, ade 2, ural, his7, tyr7, lys2, gal 1, a haploid wild type was used in all our experiments.

**Media.** The chemostat medium contained 10 g yeast extract, 10 g bactopeptone (Difco), 10 g glucose as limiting nutrient, 5 g ammonium sulphate, 2 g ammonium phosphate, 1 g magnesium sulphate and 100 mg adenine in 1 l. of distilled water at pH 5.5.

Batch cultures were grown in the following media. YEPD: containing in 1 l. of distilled water 10 g yeast extract, 20 g bactopeptone (Difco) and 20 g glucose; PROLINE: containing in 1 l. of distilled water 20 g glucose, 1.4 g yeast nitrogen base (without amino acids) and 2 g proline; YEPG: 20 ml glycerol, 10 g yeast extract and 20 g bactopeptone (Difco); ETHANOL: 20 ml ethyl alcohol (95% Merck) per l., 5 g ammonium sulphate and 0.7 g yeast nitrogen base. Proline and ethanol media also contained 0.04 g tyrosine, 0.04 g lysine, 0.04 g histidine, 0.01 g uracil and 0.1 g adenine per l., trace elements and vitamins.

**Culture conditions.** A chemostat (LH Engineering Co.) was used for continuous culture at 24 °C with glucose as the limiting nutrient. Batch cultures on different media were shaken vigorously in a waterbath at 24 °C. Batch cultures at different temperatures were made in YEPD medium shaken vigorously in a waterbath.

**Determination of cell number.** Samples were diluted with Isoton (Coulter Electronics), homogenized for 60-90 s to break up clumps and counted in an electronic particle counter (Coulter Electronics).

**Hydroxyurea treatment of asynchronous cultures.** Asynchronous cultures were divided in two and one half was diluted in fresh medium and the other was diluted in fresh medium containing hydroxyurea such that the final concentration was 0.2 M. Cell numbers were monitored at the time of incubation in hydroxyurea and subsequently to determine the percentage of cells which divide in the presence of hydroxyurea. Hydroxyurea treatment results in inhibition of DNA synthesis within 10 min at 30 °C (Slater, 1973), 23 °C (Hartwell, 1976) and 18 °C (Jagadish, unpublished observations) while RNA synthesis and protein synthesis are unaffected for at least a generation.

RESULTS

Various authors including Howell & Naliboff (1973) have shown that knowing the percentage of cells in an asynchronous culture which are past a particular event the stage of occurrence in the cell cycle of that particular event can be determined. The age distribution of cells in the cycle must be taken into account when determining the stage and time of occurrence of a particular event in the cell cycle.

Hydroxyurea rapidly inhibits DNA synthesis in yeast (Slater, 1973) and completion of DNA synthesis is necessary for cell division (Hereford & Hartwell, 1974; Hartwell, 1976). Therefore if hydroxyurea is added to an exponential culture only those cells that have completed DNA synthesis will divide in the presence of hydroxyurea. The percentage of cells in an exponential culture that divide in the presence of hydroxyurea can be used to calculate the stage in the cycle of the completion of DNA synthesis using the equation $X = 1 - \frac{\ln(N/N_0)}{0.693}$, where $X$ is the stage in the cycle of completion of DNA synthesis, $N_0$ is equal to the cell number at the time of the addition of hydroxyurea and $N$ is equal to the final cell number in the presence of hydroxyurea (Howell & Naliboff, 1973).

Yeast cells were grown in the chemostat at different steady-state growth rates, samples were taken and diluted in fresh medium at 24 °C and the percentage increase in cell number was determined. A typical experiment is shown in Fig. 1.
at a specific growth rate ($p$) of 0.264 equivalent to a mass doubling time of 2.63 h were removed from the chemostat and half the culture was diluted in fresh medium at 24 °C and the other half was diluted in fresh medium containing hydroxyurea (0.2 M final concentration). Cell numbers increased throughout the experiment in the culture without hydroxyurea but in the culture containing hydroxyurea cell numbers increased initially but then cell division ceased and cell numbers reached a plateau. Microscopic examination of the culture revealed that all cells arrested homogeneously with a bud which was the same size as the parent cell from which it was derived. These cells have a dumbbell-like appearance and were somewhat swollen because cells continue to grow in the presence of hydroxyurea although they cannot divide.

The results (Table 1) of shifting cells grown at different growth rates in the chemostat to fresh medium containing hydroxyurea demonstrate that completion of DNA synthesis does not occur at the same stage of the cell cycle at all growth rates but occurs later in the cycle at slow growth rates. If these data are converted into the time before cell division of the completion of S-phase it is apparent (Table 1) that
S-phase is completed 1.59–2.00 h before cell division in cells growing within the generation time range of 2.6 to 5.58 h. On the other hand in cells growing with a generation time of 19.25 h the time from completion of S-phase to cell division is lengthened to 2.9 h although the major expansion of the cell cycle occurs from the beginning of the cell cycle to the completion of S-phase. For instance in cells growing with a generation time of 2.6 h the time from the beginning of the cycle to the completion of DNA synthesis is 1.01 h whereas in cells growing with a generation time of 19.25 h it is as long as 16.35 h.

Similar experiments were done in which variation in nutrient composition in batch cultures was used to obtain different growth rates. Samples were removed from exponentially growing cultures, diluted in fresh medium containing hydroxyurea and the percentage increase in cell number was monitored. From the data the stage and time of occurrence of S-phase completion were determined. The results presented in

### Table 1. Effect of specific growth rate on the stage and time in the cell cycle of the completion of S-phase

<table>
<thead>
<tr>
<th>Specific growth rate, h⁻¹</th>
<th>Generation time, h</th>
<th>% increase in cell no. in presence of hydroxyurea</th>
<th>Stage in cell cycle of completion of S-phase</th>
<th>Time before cell division of completion of S-phase, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.264</td>
<td>2.6</td>
<td>53</td>
<td>0.39</td>
<td>1.59</td>
</tr>
<tr>
<td>0.206</td>
<td>3.4</td>
<td>47</td>
<td>0.44</td>
<td>1.90</td>
</tr>
<tr>
<td>0.128</td>
<td>5.4</td>
<td>29</td>
<td>0.63</td>
<td>2.00</td>
</tr>
<tr>
<td>0.124</td>
<td>5.58</td>
<td>28</td>
<td>0.64</td>
<td>2.00</td>
</tr>
<tr>
<td>0.036</td>
<td>19.25</td>
<td>11</td>
<td>0.85</td>
<td>2.90</td>
</tr>
</tbody>
</table>

Haploid wild type yeast strain A364a was grown at different steady-state growth rates in a glucose-limited chemostat at 24 °C. Samples at different specific growth rates were removed from the chemostat, diluted in fresh medium at 24 °C containing hydroxyurea (0.2 M). The percentage of cells that divided in the presence of hydroxyurea was used to calculate the stage and time in the cycle of the completion of S-phase using the equation described in the text.

### Table 2. Effect of nutrient-mediated growth-rate changes on the stage and time in the cell cycle of the completion of S-phase

<table>
<thead>
<tr>
<th>Media</th>
<th>Generation time, h</th>
<th>% increase in cell no. in presence of hydroxyurea</th>
<th>Stage in cell cycle of completion of S-phase</th>
<th>Time before cell division of completion of S-phase, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>YEPD</td>
<td>2.83</td>
<td>47</td>
<td>0.44</td>
<td>1.59</td>
</tr>
<tr>
<td>PROLINE</td>
<td>3.92</td>
<td>41</td>
<td>0.50</td>
<td>1.96</td>
</tr>
<tr>
<td>YEPG</td>
<td>6.58</td>
<td>22</td>
<td>0.71</td>
<td>1.91</td>
</tr>
<tr>
<td>ETHANOL</td>
<td>6.90</td>
<td>22</td>
<td>0.72</td>
<td>1.93</td>
</tr>
</tbody>
</table>

Haploid yeast strain A364a was grown in different media containing 0.2 M hydroxyurea in batch culture at 24 °C. The percentage of cells that divided in the presence of hydroxyurea was used to calculate the stage and time in the cycle of the completion of S-phase using the equation described in the text.
Table 2 show that DNA synthesis is completed later in the cell cycle as the mass doubling time increases. When this is converted into the time of the completion of S-phase before cell division, it is apparent that this time interval is approximately the same at different generation times (Tables 1, 2; Fig. 2B).

Variation in temperature was used to obtain different growth rates while the nutrient composition of the medium was kept constant. The mass doubling time of yeast increases as the temperature increases up to a certain point. Cells were grown at different temperatures, samples were removed and diluted in fresh medium containing hydroxyurea. The percentage increase in cell number was monitored and the stage and time of completion of S-phase in cells growing at different temperatures were determined. Table 3 shows the results obtained at different generation times. The results are in contrast to the previous ones (Tables 1, 2), in that S-phase is completed at roughly the same stage of the cell cycle at different mass doubling times.
When this is converted into time of the completion of $S$-phase before cell division, this time interval increases as the generation time increases (Fig. 2A). For instance, in cells growing with a mass doubling time of 1.96 h the completion of $S$-phase occurs 1.08 h before cell division and in cells growing with a mass doubling time of 6.5 h it is 3.32 h before cell division.

Table 3. Effects of temperature-mediated growth rate changes on the stage and time in the cell cycle of the completion of $S$-phase

<table>
<thead>
<tr>
<th>Temperature, °C</th>
<th>Generation time, h</th>
<th>% increase in cell no. in presence of hydroxyurea</th>
<th>Stage in cell cycle of completion of $S$-phase</th>
<th>Time before cell division of completion of $S$-phase, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>34.5</td>
<td>1.96</td>
<td>46</td>
<td>0.45</td>
<td>1.08</td>
</tr>
<tr>
<td>30.0</td>
<td>2.16</td>
<td>47</td>
<td>0.44</td>
<td>1.25</td>
</tr>
<tr>
<td>24.5</td>
<td>2.83</td>
<td>47</td>
<td>0.44</td>
<td>1.58</td>
</tr>
<tr>
<td>23.0</td>
<td>3.30</td>
<td>44</td>
<td>0.47</td>
<td>1.77</td>
</tr>
<tr>
<td>20.0</td>
<td>4.00</td>
<td>47</td>
<td>0.44</td>
<td>2.44</td>
</tr>
<tr>
<td>19.0</td>
<td>4.90</td>
<td>44</td>
<td>0.47</td>
<td>2.60</td>
</tr>
<tr>
<td>17.5</td>
<td>6.50</td>
<td>41</td>
<td>0.49</td>
<td>3.32</td>
</tr>
</tbody>
</table>

Haploid yeast strain A3644 was grown in YEPD media in batch culture at different temperatures. Samples were removed from the cultures and diluted in fresh media containing hydroxyurea (0.2 M). The percentage of cells that divided in the presence of hydroxyurea was used to calculate the stage and time in the cycle of the completion of $S$-phase using the equation described in the text.

**DISCUSSION**

There are very few studies of the yeast cell cycle where the length of the cell cycle time is altered by environmental conditions although such studies in bacteria (Cooper & Helmstetter, 1968; Helmstetter & Cooper, 1968) and in higher eukaryotes (see Mitchison, 1971; Prescott, 1976) have contributed to our understanding of the control of cell division in these organisms.

In the present series of experiments we have determined the latest time in the cell cycle that addition of hydroxyurea, an inhibitor of DNA synthesis, will prevent cell division. As hydroxyurea rapidly inhibits DNA synthesis in yeast (Slater, 1973) and completion of $S$-phase is necessary for cell division (Hereford & Hartwell, 1974; Hartwell, 1976) we conclude that only those cells that have completed $S$-phase at the time of addition of hydroxyurea will divide in the presence of this inhibitor. We therefore have used hydroxyurea to determine the stage and time in the cycle that $S$-phase is completed when the growth rate of $S. cerevisiae$ is varied by alterations in either the medium composition or the temperature of cultivation.

We have observed that when $S. cerevisiae$ was grown at different steady-state specific growth rates in a chemostat by alteration in the flow rate of the limiting nutrient glucose, the stage in the cycle at which completion of $S$ occurs is dependent on growth rate (Table 1). However, when stage in the cycle is converted into the time before cell division that $S$-phase is completed it appears that except at extremely slow growth rates this time is almost independent of growth rate. This indicates
The cell cycle of S. cerevisiae

that at slow growth rates expansion of cell cycle time is primarily a result of the increased time a cell takes from division to the completion of S-phase. Similar results were obtained when growth rate was varied by alterations in medium composition in batch cultures (Table 2). No data exist on the effect of hydroxyurea on DNA synthesis at slow growth rates. However our observation that the percentage increase in cell numbers is reduced at slow growth rates is an indication that hydroxyurea inhibits DNA synthesis rapidly at slow growth rates. If our results had shown that more cells divided in the presence of hydroxyurea at slow growth rates than fast growth rates this might indicate that hydroxyurea did not result in a rapid inhibition of DNA synthesis at slow growth rates and cells undergoing DNA synthesis when hydroxyurea was added were able to complete DNA synthesis and subsequently divide. While our results indicate that the length of $G_1 + S$ expands at slow growth rates this expansion may be entirely a feature of expansion of the $G_1$ phase of the cycle; $S$-phase may not expand. Indeed, Barford & Hall (1976) have grown yeast on 3 different media resulting in mass doubling times of $1.54$, $3.08$ and $7.25$ h and shown that in these cells the length of $S$-phase is $45.5$, $46.0$ and $47.8$ min, respectively.

The present results suggest that the cell cycle of yeast is similar to mammalian cells where the length of the $G_1$ phase of the cycle varies with cycle times while the $S + G_2 + M$ time is almost independent of cycle time when the generation time is varied by alteration in media composition or alterations in the pH of the medium (see Mitchison, 1971; Prescott, 1976).

Rather different results were observed when growth rate was varied by alterations in temperatures of cultivation. Completion of $S$-phase occurred at approximately the same fractional stage of the cycle when mass doubling times ranging from $1.96$ to $6.5$ h were obtained by alterations in temperature of cultivation (Table 3). Thus the time from $S$-phase to cell division expands as the mass doubling time is increased as does the time from division to completion of $S$-phase. These findings are similar to those of Rao & Engelberg (1965) who found that temperature of cultivation in HeLa cells affected cell cycle time by modifying all phases of the cell cycle to a similar extent. In contrast, Sisken (1963, 1965) and Sisken, Morasca & Kibby (1965) working on human amnion cells and Watanabe & Okada (1967) working on mouse leukaemic cell line 15178y observed that while the lengths of all phases of the cycle were modified by temperature the major expansion was in the length of the $G_1$ phase. It is not perhaps surprising that increasing the growth rate by elevating the temperature of cell cultivation results in a contraction of all phases of the cycle. Elevated temperatures will lead to increased synthesis and activities of compounds associated with cell division processes. The fact that changes in growth rate associated with variations in medium composition selectively alter the length of $G_1$ is more difficult to explain. There is evidence (Hartwell, 1974) that there is a control event in $G_1$ (prior to budding and the initiation of DNA synthesis) which cells can only traverse if they have sufficient nutrients to complete the entire cell cycle. In addition Johnston, Pringle & Hartwell (1977) found that when cells with a small bud were starved for nitrogen they could complete a cell cycle without a significant accumulation of protein. If the decision to embark on the $S + G_2 + M$ phases of the cycle is not taken...
unless at all growth rates cells have adequate nutrients to complete these phases, then the observation that cells complete these phases in a time independent of growth rate is not unexpected. Similar results are obtained in *Escherichia coli* where the time from initiation of DNA synthesis to cell division is constant at mass doubling times from 20 to 60 min.

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REFERENCES


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