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Cell Biology

Nature of the G1 phase of the yeast *Saccharomyces cerevisiae*

(cell cycle/hydroxyurea)

R. A. SINGER AND G. C. JOHNSTON

Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia B3H 4H7 Canada

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ABSTRACT **Under conditions that protract the S phase for *Saccharomyces cerevisiae* without affecting steady-state rates of cell growth or proliferation, there were striking decreases in the length of the G1 period. These decreases were localized in the period between mitosis and the start event that initiates a new cell cycle. We conclude that this major fraction of the G1 period has no functional role in the DNA-division sequence of cell cycle events.**

The cell cycle involves the periodic replication of DNA and segregation of replicated DNA with cellular constituents to progeny cells. Mitchison (1) has described the eukaryotic cell cycle by a model composed of two independent cycles, the DNA-division cycle and the growth cycle. The DNA-division cycle consists of events concerned with the replication and segregation of DNA. The growth cycle, on the other hand, is a more loosely defined concept, normally used to describe processes that provide the bulk of the new cytoplasm. The performance of these two cycles is independent (1), but cells have mechanisms so that repeated DNA-division cycles do not outstrip the process of growth. This coordination generally occurs in the G1 period of the cell cycle, after mitosis (M phase) is completed but before DNA replication (S phase) is initiated (for review, see ref. 2).

Much is known about the regulation of the cell cycle of the yeast *Saccharomyces cerevisiae* (3). For example, one event in the DNA-division cycle requires growth to a critical size for its completion (2–4). This point of regulation has been referred to as "start" and lies temporally near the end of the G1 period (2). The G1 period of the cell cycle also is (for other eukaryotes as well) the most sensitive to growth conditions because the combined length of the cell cycle periods S + G2 + M generally remains constant under varied growth conditions (5–9). As nutritional conditions are changed so that growth rates are increased, the time in G1 is correspondingly decreased.

It is generally held that, throughout the eukaryotic G1 period, a sequence of cell cycle-specific events must occur in preparation for DNA synthesis (1, 10, 11). Recently this view has been questioned. Both Cooper (12) and Liskay *et al.* (13) have theorized that G1 is present in eukaryotic cells, not because events specific to G1 are taking place but because events specific to growth have not yet occurred in sufficient quantity. In this view, G1 is present in cells solely to allow accumulation of sufficient "division potential" to initiate a new S phase.

This alternative view of the nature of the G1 period is based upon two types of observations. First, Cooper has developed his hypothesis by extending the model for bacterial cell division. Bacteria have the ability to initiate a new round of chromosome replication (a new DNA-division cycle) when sufficient mass has accumulated, even prior to the completion of previously initiated rounds of replication. Thus, cell cyles in bacteria can overlap (14). In this view of the cell cycle, an interval separating the initiation of a new DNA-division cycle from the preceding cell division event is simply a reflection of an insufficient accumulation of mass during the previous cell cyle. Second, Liskay and coworkers have described Chinese hamster cells that have no discernible G1 period (13, 15, 16). This observation has led them to suggest that G1 need not be an integral part of the eukaryotic cell cycle.

Whether the G1 period is (*i*) necessary for the execution of a sequence of cell cycle-specific events or (*ii*) simply part of a larger period for growth, altering the growth rate would be expected to alter the length of G1 in much the same way. In contrast, the possibility of decreasing the rate of performance of DNA-division-cycle events, without affecting overall growth rate [a procedure used for cell cycle studies in bacteria (17)], leads to quite different predictions for G1. For example, if G1 is an obligatory part of the cell cycle, reflecting the need for G1-specific events, then slowing the rate of progression of some other aspect of the DNA-division cycle without affecting overall growth rate should not markedly alter the G1 period. If, however, G1 simply represents a period of ongoing growth, then (because growth and cell division are independent processes) slowing the rate of progression of some aspect of the DNA-division cycle should allow greater than normal growth during the protracted performance of these events and should result in a shorter than normal G1 period. Because such experiments may be conducted under identical growth (nutritional) conditions, slowing the DNA-division cycle should clearly distinguish between the two views of the G1 period previously described.

To this end, with *S. cerevisiae* we have used procedures that affect the rate of progression through S phase. Under identical nutritional conditions and without affecting the overall rate of cell number increase or growth, we find that slowing the rate of progress through the DNA-division sequence causes a striking decrease in the time spent in the G1 period. Thus, in this communication we provide evidence that in an organism that normally exhibits a significant G1 period, most of this G1 is not an obligatory part of the cell cycle.

MATERIALS AND METHODS

Strains and Media. The haploid strain GR2 (*a ura1 his6*) (ATCC 42564) has been described (18). Strain 13052 that carries the temperature-sensitive allele *cdc*8-3 (19) and strain 428 that carries *cdc*13-1 (20) are both derivatives of strain A364A and were provided by L. H. Hartwell. Strain GR150 is a *cdc*8 strain constructed to be isogenic to strain GR2. Cells were grown at room temperature in the complex medium YM1 (21). Where appropriate, hydroxyurea was added to 1.5 mg/ml from a stock solution of 20 mg/ml in YM1. The mating pheromone α factor was prepared by the method of Bücking-Throm *et al.* (22).

Analysis of Cellular Parameters. Cell number was determined with an electronic particle counter (Coulter Electronics,

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Hialeah, FL). Prior to counting, cells were either sonicated briefly (23) or treated with the enzyme glusulase to separate cells that had undergone cytokinesis (24). Execution point was determined by increase in cell number as described (8).

RESULTS

Three procedures were employed to slow the progress of cells through the DNA-division cycle. Each of these procedures involved affecting the rate of progress through S phase.

Effect of Hydroxyurea on Cell Cycle Progression. The first procedure employed the DNA synthesis inhibitor hydroxyurea. A high concentration of hydroxyurea causes arrest of cells in S phase (25). At lower concentrations of hydroxyurea, we found rather different effects on the progression of cells through the cell cycle. Hydroxyurea-treated cells, after an initial lag period, grew at the same 2.7- to 2.8-hr generation time as untreated cells. The rate of growth (cell-mass increase) must be unaffected by these conditions because the normal rate of cell number increase of these hydroxyurea-treated cells could continue indefinitely (data not shown). We continually subcultured cells for several days in the presence of hydroxyurea before performing subsequent experiments.

A low concentration of hydroxyurea was used to retard but not to block progress through S phase. Because of the difficulty in measuring the length of S phase in yeast, no direct measurements were made. However, to ensure that S phase was protracted by hydroxyurea treatment, we examined the effect of hydroxyurea on the timing of completion of cell cycle steps. Certain mutations, the *cdc* mutations, affect cell cycle progress, and may be used to define events that function at specific points in the cell cycle. The last point in the cell cycle affected by a *cdc* mutation is referred to as the execution point of that mutation [execution point is operationally defined as that point in the cell cycle past which a cell is no longer sensitive to nonpermissive conditions (26)]. We examined the execution point of the *cdc*8 mutation, which affects DNA synthesis (27), and found that hydroxyurea treatment moved completion of this step from 0.37 to 0.56 of the yeast cell cycle. Another *cdc* mutation, *cdc*13, defines a step normally completed at the end of S phase (20). In similar fashion, execution point determination showed that hydroxyurea treatment moved completion of the *cdc*13 step from 0.35 to 0.50 of the cell cycle. Thus, without affecting the rate of cell number increase, hydroxyurea treatment lengthens the period encompassing S phase.

Upon hydroxyurea treatment, the proportion of unbudded cells fell from 45% to 15% (Fig. 1). Because the proportion of unbudded cells usually approximates the proportion of cells in G1 period (7, 9), these results show that, without affecting the rate of cell number increase, hydroxyurea treatment shortens the unbudded period of the cell cycle and, thus, may shorten the G1 period.

Effect of Hydroxyurea on Length of G1. To demonstrate that the decreased proportion of cells without buds also indicated a shorter length of time in the G1 period prior to the initiation of new cell cycles, we examined the effect of hydroxyurea treatment on the timing of another DNA-division-sequence event, start. Start is the earliest known gene-mediated step in the DNA-division sequence (3). Before commitment to a new DNA-division sequence by the completion of start, cells of the *a* mating type are still sensitive to the mating pheromone α factor (28). We determined the point of loss of α-factor sensitivity (the execution point) for both hydroxyurea-treated and untreated populations of cells of strain GR2. In the untreated population, these cells executed the α-factor sensitive step (start) at approximately 0.30 of the cell cycle, measured from cell separation. In contrast, cells growing in the presence of hydroxyurea

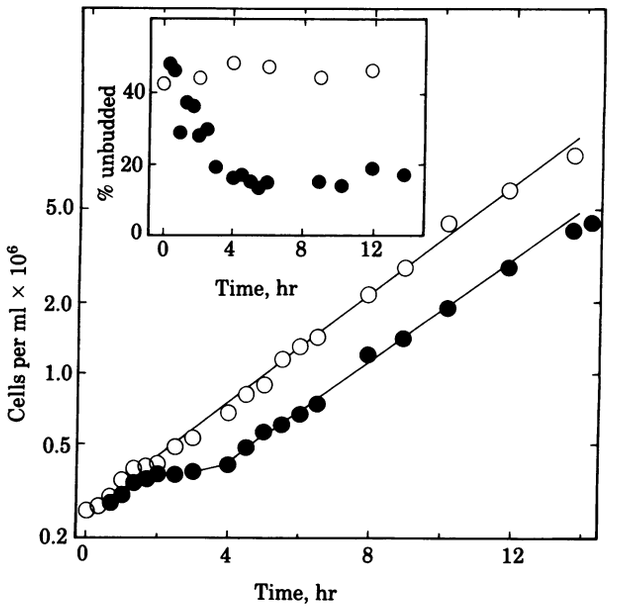

FIG. 1. Effect of hydroxyurea on cellular parameters. Cells of strain GR2 were grown in the complex medium YM1. At time 0, hydroxyurea was added to 1.5 mg/ml. Cell concentrations and the percentage unbudded were determined as described. ●, With hydroxyurea; ○, without hydroxyurea.

executed start at 0.0 of the yeast cell cycle. Thus, not only is the unbudded period shortened in the presence of hydroxyurea, but cells are also able to execute the first step in the DNA-division sequence immediately after completion of cell separation.

Normally yeast cell number determinations are made after brief sonication to separate cells that have completed both the cytokinesis and cell separation steps but have failed to completely detach. Thus, the usual procedure with mild sonication really only allows the measurement of execution point relative to cell separation. Conceivably during hydroxyurea treatment, the period between cytokinesis and cell separation may expand in a manner compensatory to the decrease in the unbudded period. To examine this possibility, we measured execution points relative to the cytokinesis process. This can be accomplished by using the enzyme mixture glusulase to separate cells by cell wall digestion (24). When glusulase treatment was employed instead of sonication, we found that here too the execution point of strain GR2 for α-factor sensitivity was moved by hydroxyurea treatment to 0.05 of the cell cycle.

The G1 period may be thought to begin not with cytokinesis but after mitosis. Consequently, the shortened G1 period between cytokinesis and start brought about by hydroxyurea treatment may result simply from a large compensatory expansion of the normally short interval between mitosis and cytokinesis. To test this hypothesis, we determined the proportion of GR2 cells that had two nuclei and, thus, had completed mitosis but had not yet undergone cell separation, which follows cytokinesis. After fixation and sonication to separate cells, staining with Giemsa (29) showed that 6.6% of >1500 hydroxyurea-treated cells and 5.7% of >1000 untreated cells were in the interval between mitosis and cell separation. This finding of a constant interval between mitosis and cell separation, coupled with the demonstration that cytokinesis immediately precedes start, shows that hydroxyurea treatment does in fact shorten the period between mitosis and start.

Effect of Hydroxyurea on Bud Initiation of Mother and Daughter Cells. Because of the budding mode of division of *S. cerevisiae*, a daughter cell is usually smaller than a mother cell

and must grow to a greater extent prior to initiation of its own bud. Thus, the cell cycle of a mother cell is usually shorter than the cell cycle of its daughter (6). This type of behavior has been described by a mixed mother–daughter model of cell division (7). If this model of yeast division is also valid for growth in the presence of hydroxyurea (when execution of the start event occurs immediately after cytokinesis) then, because execution point measures a population average, this might indicate that some cells execute start well after cytokinesis, whereas others may execute start in the previous cell cycle. However, during steady-state growth in the presence of hydroxyurea, both mother and daughter cells are larger than normal and of nearly equal sizes. Under these conditions a mixed mother–daughter model of the yeast cell cycle may not be valid, and cells may be better described by a homogenous mother–daughter model (7), in which both mother and daughter have the same generation times. Time-lapse photography of a limited number of cells of strain GR2 growing in the presence of hydroxyurea showed that both mother and daughter cells tended to initiate new buds simultaneously. This would suggest that cells growing in the presence of hydroxyurea exhibit division patterns characteristic of the homogenous mother–daughter model of cell division and that execution of the start event does indeed occur for all cells immediately after mitosis and cytokinesis.

Effect of Trenimon on Cell Division. A second procedure for slowing the DNA-division sequence was use of the DNA synthesis inhibitor Trenimon (30, 31). At approximately 0.3 mg/ml this inhibitor had the same cellular effects as hydroxyurea did (data not shown).

Effect of Intermediate Temperatures on Strains Bearing the *cdc*8 Mutation. To circumvent any problem of possible secondary effects caused by DNA synthesis inhibitors, a third procedure was used. This procedure made use of the temperature-sensitive mutation *cdc*8 (19, 27). When placed at the nonpermissive temperature of 36°C, cells of strain GR150, isogenic to strain GR2 but bearing the *cdc*8 mutation, exhibited within one cell cycle an S-phase arrest, characteristic of this mutation. In contrast, at the intermediate temperature of 26°C, the rates of cell number increase of both the wild-type strain (GR2) and the mutant strain (GR150) were similar. Under these latter growth conditions, although the rates of cell division were essentially the same, the proportion of unbudded cells was much smaller in the mutant population, 12% compared to 45% for the wild type. (At the permissive temperature of 22°C, strain GR150 had 35% unbudded cells.) Again, when we determined the execution point for α-factor sensitivity, we found that, at the intermediate temperature, the strain bearing the *cdc*8 mutation executed start at approximately 0.05 of the yeast cell cycle (compared to 0.30 for wild-type cells growing at the same temperature and to 0.30 for mutant cells growing at 22°C).

DISCUSSION

We employed three separate procedures to protract the S phase of the yeast cell cycle. In each case there was a striking decrease in the length of the G1 period without any effect on the overall rate of cell number increase. These experiments suggest that the length of the G1 period is responsive to the relative rate of progression of the DNA-division sequence in addition to growth conditions. Decreasing the rate of the DNA-division-sequence progression without significantly affecting the rate of growth is correlated with shorter G1 periods. This correlation may be better understood in the following way. For cells slowly progressing through the DNA-division sequence, a larger proportion of the total growth required to reach the critical size may be achieved before the end of mitosis. Under these conditions, a smaller proportion of total growth will remain to be completed in G1 before start, and a shorter period of time in G1 will be required to do so. Thus, a change in the length of G1 between cytokinesis and the start event probably reflects a change in the proportion of total growth remaining after cytokinesis to be completed before start.

This suggestion that G1 is nothing more than a period of growth is consistent with the large body of work for yeast. Many studies have shown that the length of the G1 period is variable in response to different growth conditions (6, 7, 9). Moreover, the same appears to be true for animal cells (reviewed in ref. 10). A particularly illustrative example has been provided by Liskay *et al.* (32), who showed that, for an animal cell line devoid of a measurable G1 period, a significant G1 period could be induced by simply slowing the rate of protein synthesis.

The results presented here do not absolutely rule out the possibility that G1-specific events still occur before start but much more rapidly under the conditions described. However, it must be emphasized that in no case were nutritional conditions altered and in no case was the steady-state rate of cell number increase affected. Thus, to explain the more rapid completion of such G1-specific events, increased growth rates cannot be invoked.

Results from preliminary experiments, in which higher hydroxyurea concentrations led to decreased steady-state growth rates, also argue against the existence of extensive prestart, G1-specific events. In these experiments the execution point for start, as determined by α-factor sensitivity, was −0.2 of the yeast cell cycle relative to cell separation (unpublished data). (This value should be compared to the control value of +0.3 found in the absence of hydroxyurea for the same strain under the same nutritional conditions.) A qualitatively similar execution point result was found before (8) but in a manner differing significantly from that just described. First, in those experiments nutritional conditions were varied to generate different growth rates; at the most rapid growth rate, a negative value for the execution point of start was found. Second, in those experiments the execution point was determined for a temperature-sensitive mutation thought to be leaky (28); unfortunately, this suggestion of an incomplete block at the restrictive temperature creates uncertainty regarding the measurement's quantitative value. [Execution point determinations are sensitive to the rate at which an intracellular cell cycle-blocking state arises after imposing a particular arresting condition (33).] Nevertheless, our results do suggest that at least one early cell cycle event seems to occur in the previous cell cycle, and, indeed, cell cycles may be able to overlap to some extent. A number of events in the yeast cell cycle may be shifted in this way

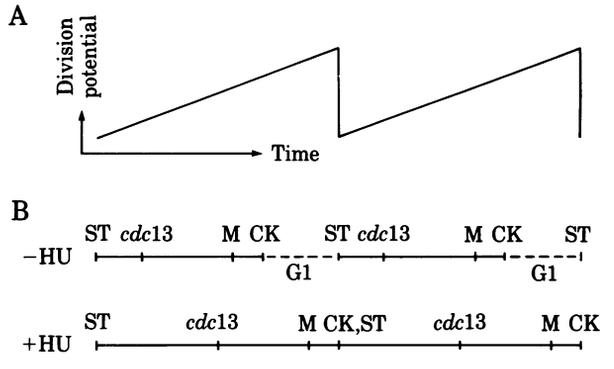

FIG. 2. Schematic representation of cell division relationships over two divisions. (*A*) Accumulation of "division potential" with time. (*B*) Timing of cell cycle events with and without hydroxyurea. The time scale is the same as in *A*. ST, Start; *cdc*13, execution point of the *cdc*13 mutation; M, mitosis; CK, cytokinesis; HU, hydroxyurea.

to determine the extent of cell cycle overlap.

The finding that start can be executed at 0.0 of the yeast cell cycle is supported by our observation that for both mother and daughter cells bud initiation occurs at roughly the same time under these steady-state growth conditions. This result was anticipated by earlier work of Yamata and Ito (34), who used similar hydroxyurea concentrations in an attempt to synchronize the budding mode of mother and daughter cell pairs. Although they only looked at the first few divisions after hydroxyurea addition, they too found that, in the presence of hydroxyurea, bud initiation was simultaneous on both mother and daughter cells.

In the absence of genetic evidence for any prestart steps, we suggest that a prestart G1 period is merely a manifestation of a block at the start event of the DNA-division sequence caused by insufficient growth. This view suggests that the G1 period need not be considered an extended sequence of cell cycle specific events. There need be no interval at all between the end of mitosis and cytokinesis and the execution of start. Although temporally the start event may be completed near the end of the G1 time interval, functionally (with respect to the cell cycle) start may initiate the pre-S phase. We further agree with the suggestion by Cooper (12) that use of the term cycle may not be appropriate. The events that make up cell division are better described as a sequence as shown in Fig. 2, with a beginning (completion of start) and an end (completion of mitosis). We would suggest that the bulk of the G1 period we observe as a temporal entity is in fact without functional significance with respect to the DNA-division sequence.

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