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Cell Cycle-Dependent Expression of Thymidylate Synthase in *Saccharomyces cerevisiae*

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Synchronous populations of *Saccharomyces cerevisiae* cells, generated by two independent methods, have been used to show that thymidylate synthase, in contrast to the vast majority of cellular proteins thus far examined, fluctuates periodically during the *S. cerevisiae* cell cycle. The enzyme, as assayed by two different methods, accumulated during S period and peaked in mid to late S phase, and then its level dropped. These observations suggest that both periodic synthesis and the instability of the enzyme contribute to the activity profile seen during the cell cycle. Accumulation of thymidylate synthase is determined at the level of its transcript, with synthase-specific mRNA levels increasing at least 10-fold to peak near the beginning of S period and then falling dramatically to basal levels after the onset of DNA synthesis. This mRNA peak coincided with the time during the cell cycle when thymidylate synthase levels were increasing maximally and immediately preceded the peak of DNA synthesis, for which the enzyme provides precursor dTMP.

DNA synthesis is precisely regulated in yeasts in a cell cycle-dependent manner, with a periodic burst of synthesis in what defines the S phase of the cycle (36). The rate of overall RNA and protein synthesis, however, is constant throughout the cell cycle (reviewed in reference 18) as is the rate of accumulation of most of the abundant protein species (17, 31). Of a total of 900 proteins examined, only 8 appeared to be periodically synthesized, including histones H2A, H2B, and H4 (26, 31). To understand the molecular events which govern progress throughout the cell cycle, it is necessary to identify and study those few proteins which are periodically expressed.

Thymidylate synthase (EC 2.1.1.45) regulates the only de novo route of dTMP biosynthesis (29), and dTMP generally has no fate in nucleic acid metabolism other than as a DNA precursor. It is therefore not surprising that in several eucaryotic systems studied to date, thymidylate synthase activity is elevated in rapidly dividing cells, such as in regenerating liver (32), embryonic (40), and tumor tissues (28). Furthermore, the enzyme accumulates in S phase in several synchronized cell types (2, 38, 42). In addition, alterations in intracellular pools of dTMP have been implicated in thymineless death (16, 27, 30) and the control of recombination (19) and mutation (3). These observations imply that thymidylate synthase is precisely regulated and suggested to us that the enzyme may represent one of those rare proteins in yeasts whose expression is cell cycle dependent.

Saccharomyces cerevisiae provides the following advantages for examining the pattern of thymidylate synthase accumulation during the cell cycle and for elucidating the mechanism(s) responsible for its regulation. (i) Synchronous populations of cells are easily generated. (ii) The yeast gene encoding thymidylate synthase (*TMP1*) has been previously isolated (45). (iii) Mutant alleles (both temperature sensitive and nutritional) of the *TMP1* gene have been isolated, and methods have been developed for generating additional mutants (10, 30). (iv) Temperature-sensitive mutants affecting DNA synthesis and progress throughout the cell cycle are available (23). (v) Yeast thymidylate synthase has already been purified and characterized (9).

The observations reported here show that thymidylate synthase, like yeast histones, is synthesized in a periodic fashion, accumulating during the S period. It would therefore appear that *S. cerevisiae* proteins specifically involved in chromosome replication form a special class whose regulation is intimately linked with DNA synthesis.

MATERIALS AND METHODS

Strains and plasmids. The plasmids pTL1 (45) and YIp5 (44) were used, respectively, as sources of *TMP1* and *URA3* gene sequences in the hybridization experiments. pTL221 and pBTAH were used in thymidylate synthase expression studies in *Escherichia coli*. pTL221 consists of the yeast *TMP1* gene on a 3.6-kilobase (kb) *Hin*dIII-*Bgl*II fragment cloned into pBR322, whereas pBTAH comprises the *E. coli thyA* gene on a 1.2-kb *Hin*dIII fragment in pBR322 (4). *S. cerevisiae* strains used were S288C (α *gal-2 mal*$^-$ *mel*$^-$ *SUC-2 CUP-1*) from the Yeast Genetic Stock Center, AH22 (**a** *leu2-3 leu2-112 his4-419*) from G. Fink, SKQ2N (**a**/α *ade1*/+ +/*ade2* +/*his1*) from C. S. McLaughlin, and FH4C (37). *E. coli* Rue10 is a *thyA* derivative of HB101 (from G. Wilson). Plasmid DNA was isolated as described by Clewell and Helinski (15) and modified by Birnboim and Doly (6).

Media. The complex and minimal media for growth of *S. cerevisiae* were as described previously (43). The 0.1% medium is described by Hereford et al. (26). Growth of *E. coli* was in TBYET medium (5).

Generation of synchronous populations of *S. cerevisiae*. Separation of cells of strain SKQ2N according to cell cycle stage by centrifugal elutriation was performed as previously described (17, 21). The quality of cell separation by elutriation was assessed by scoring at least 100 DAPI-stained cells from each fraction for characteristic morphological types (17, 26). DNA content was determined by diphenylamine assay (12). Cell pellets of fractions corresponding to different cell cycle stages were stored at −80°C and used for the various thymidylate synthase assays (see below) and for

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assay of invertase (β-fructofuranosidase; EC 3.2.1.26) activity (14, 20).

Synchronization of *S. cerevisiae* AH22 by α-factor treatment was essentially as described by Hereford et al. (26). At various times during synchronous growth, samples (10 to 40 ml) were transferred to 50-ml centrifuge tubes containing 5 ml of crushed ice and centrifuged at 4°C for 5 min. The pellets were frozen in liquid nitrogen and stored at −80°C. These samples were then used as a source of material for determining DNA content, thymidylate synthase activity, 5-fluorodeoxyuridylate (FdUMP)-binding capacity, and acid phosphatase activity.

Thymidylate synthase assay. Thymidylate synthase was assayed by the radiochemical procedure measuring tritium release from [5-^{3}H]dUMP, as modified by Bisson and Thorner (7). Pellets of frozen elutriator-separated samples were thawed in 2 to 3 volumes of ice-cold buffer A (20% glycerol, 10 mM 2-mercaptoethanol, 1 mM EDTA, 20 mM Tris-hydrochloride [pH 7.5]). Acid-washed glass beads (0.5-mm diameter) were added to below the meniscus, and the suspension was vigorously mixed at 4°C (five 30-s bursts on a vortex mixer). After centrifugation, the supernatant fraction was removed from the disrupted cells and assayed for enzyme activity. Frozen α-factor-synchronized cells were thawed in 100 μl of buffer A (4°C). Brij 58 was then added to the cell suspension to a final concentration of 3% to permeabilize the cells (25), and thymidylate synthase activity per milliliter of culture was determined by assaying activity in 10-, 25-, and 40-μl samples. The resulting data were plotted, and a curve was fitted by regression analysis to determine each data point.

FdUMP-binding assay. The FdUMP-binding assay is based on detection and quantitation on polyacrylamide gels of the ternary complex formed from thymidylate synthase, N^5,N^{10}-methylenetetrahydrofolate, and radiolabeled FdUMP (5). Samples of [6-^{3}H]FdUMP-treated crude cell extracts, containing 25 μg of soluble protein, were boiled in sodium dodecyl sulfate-containing gel sample buffer and separated on a 12.5% sodium dodecyl sulfate-polyacrylamide gel as previously described (5). The radioactive bands were visualized by fluorography, and the fluorograms were quantitated by densitometry with a Beckman model CDS-200 scanning spectrophotometer.

DNA content determination. Total DNA content of samples was determined by using a threefold miniaturization of the diphenylamine assay of Burton (12) as modified by Abraham et al. (1) and Richards (41).

Acid phosphatase assay. Frozen samples obtained from synchronous cultures grown as described above (i.e., high phosphate, conditions which repress acid phosphatase in yeasts) were assayed by the method of Bisson and Thorner (8) with the following modification. Frozen samples containing cells from 3 ml of culture were thawed and suspended in 100 μl of 50 mM sodium citrate buffer (pH 3.5)–1% Brij 58–1% Triton X-100. Each data point presented was obtained by regression analysis of activity measurements performed on 10, 25, and 40 μl of the suspensions.

RNA isolation, separation, transfer, and hybridization. Samples (20 ml) from α-factor-synchronized cells were added to 1 ml of 0.1 M sodium azide, and RNA was isolated as described by Carlson and Botstein (13). Total yeast RNA was separated by glyoxal gel electrophoresis by the method of McMaster and Carmichael (35) and transferred to nylon membranes (Pall Corporation) by the method of Thomas (46). A 1.2-kb *Hin*dIII to *Eco*RI fragment of plasmid pTL1, containing nearly the entire yeast *TMP1* gene, was isolated from acrylamide gels by the method of McDonnell et al. (34). The fragment was labeled with [^{32}P]dCTP (Amersham Corp.) by nick translation with *E. coli* DNA polymerase I (33). Hybridization conditions were those described by Thomas (46). The blots were allowed to expose Curex film at −80°C, using Du Pont intensifier screens. The autoradiograms were quantitated by scanning with a Unicam SP8-100 spectrophotometer adapted for densitometry.

RESULTS

Thymidylate synthase levels are dependent on cell cycle stage in several eucaryotes, with maximal levels occurring in S phase. To determine whether thymidylate synthase represents one of the rare yeast proteins which is periodically expressed, we used two different assay methods to follow enzyme levels in synchronous populations of yeast cells obtained both by centrifugal elutriation and by α-factor synchrony.

Tritium-release assay for thymidylate synthase during the cell cycle. The periodic expression of thymidylate synthase in soluble extracts of strain SKQ2N is shown in Fig. 1. Here, cells in balanced logarithmic growth were separated in an elutriator rotor according to cell cycle stage, and thymidylate synthase activity was assayed by ^{3}H release from the substrate [5-^{3}H]dUMP. The quality of the separation of cells according to their position in the cell cycle was assessed after elutriation by a morphological analysis of cell types and by determination of DNA content (Fig. 1a). Fractions 2 to 5, over which DNA content almost doubled and unbudded cells were mostly replaced by cells with small buds, correspond to S phase. In such a typical separation, thymidylate synthase activity rose from the low value of 15 to 20 μU/mg in fraction 1, representing G1, to roughly three times basal levels and reached its peak around mid-S phase (Fig. 1b). After the S period, enzyme activity dropped to near basal levels. In contrast, total invertase activity showed no increase during S phase.

When assaying activity in situ in permeabilized populations of synchronized *S. cerevisiae* cells generated with the yeast pheromone α-factor, it was also necessary to monitor the degree of cell synchrony. Cells were arrested in G1 phase with α-factor, and when 90% of the cells were without buds, indicating they were in G1, α-factor was removed. The population then underwent at least one round of synchronous growth, resulting in the synchronous appearance and then disappearance of cells with small buds (Fig. 2). The proportion of cells with small buds began to increase between 10 and 20 min after release and peaked at 40 min. DNA synthesis in synchronous cultures also began between 10 and 20 min and reached a plateau at ca. 40 min (Fig. 2). Therefore, *S. cerevisiae* AH22 behaved typically after release from α-factor arrest, and DNA synthesis was coincident with budding (48).

Thymidylate synthase and acid phosphatase activities were assayed in samples collected from the same synchronous culture. To be able to estimate the total amount of enzyme activity per milliliter of culture, we chose to assay activity in situ with Brij 58-permeabilized whole cells. The activity of thymidylate synthase began to increase less than 10 min after release and continued to increase until 40 min. This rise in activity began close to the beginning of S phase and reached a maximum toward the end of S phase. The synthase activity then decreased. The activity of acid phosphatase, on the other hand, increased continuously (Fig. 2), as would be expected for an enzyme which is not cell cycle regulated.

FdUMP binding during the cell cycle. We also determined the amount of thymidylate synthase by measuring binding to FdUMP, which complexes covalently and stoichiometrically to thymidylate synthase in the presence of N^5,N^{10}-methylenetetrahydrofolate. When elutriator-separated cell fractions were assayed by this method (Fig. 3), the maximum enzyme level coincided with DNA synthesis. The binding maximum was fourfold elevated over values at the beginning and end of the cell cycle. When synchronized cultures of AH22 were assayed for synthase levels by this method (Fig. 4b), values per milligram of protein increased between 10 and 40 min and then decreased from 40 to 60 min during the G2 and M phases. Levels began to increase again before the second S period.

The parallel increase and decrease of thymidylate synthase during synchronous growth, as measured by the ^3H-

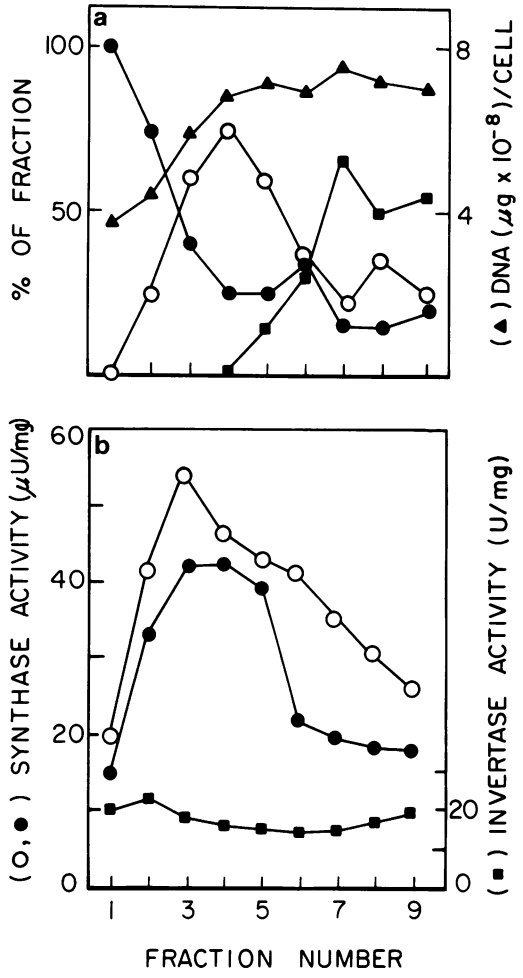

FIG. 1. Periodic thymidylate synthase activity in elutriated cells. (a) Cells separated by centrifugal elutriation scored microscopically for cell types (unbudded cells, ●; cells with small buds, ○; cells with large buds, ■) and by diphenylamine assay for DNA content (▲). Cells with small buds were uninucleate, whereas those scored as having large buds had migrating nuclei or were binucleate. The unbudded cells in fraction 1 were in G1, whereas S phase spanned fractions 2 to 5. Nuclear migration followed the completion of DNA synthesis (fractions 7 and 8), with cell separation occurring in the final fraction. (b) Thymidylate synthase activity in cell extracts of the nine fractions as assayed by ^3H release and total invertase activity as assayed by the two-step colorimetric method (14, 20). Solid symbols represent assays performed on fractions from the same rotor run. Open symbols for synthase activity are from a different separation and correspond to the microscopic analysis in (a) and the data in Fig. 3; (b) reflects the range of variation seen with synthase assays performed on different elutriator separations. The data points represent an average of at least two assays.

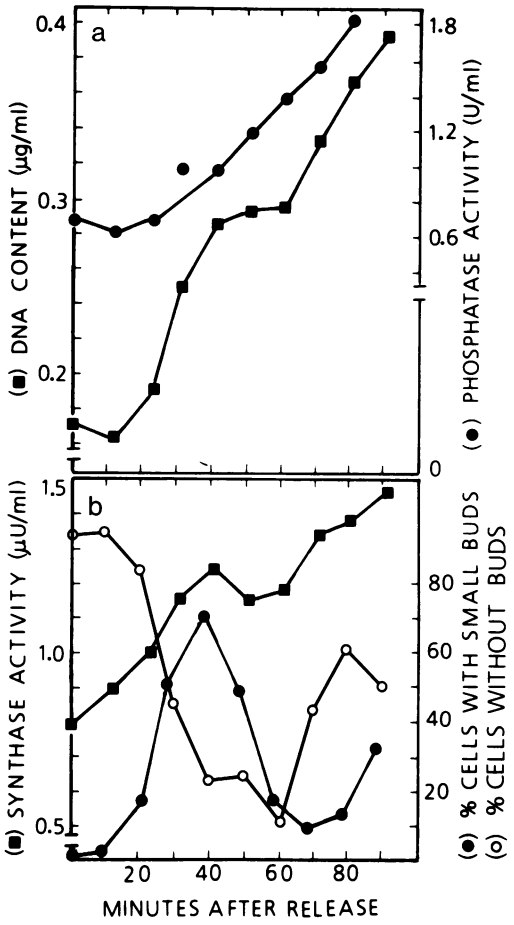

FIG. 2. DNA content, acid phosphatase activity, and thymidylate synthase activity in α-factor-synchronized cultures. Cells were synchronized with α-factor, and samples were removed at 10-min intervals. (a) Acid phosphatase levels as determined colorimetrically in permeabilized cells and DNA content as determined by the diphenylamine assay. (b) Proportion of cells with small buds and cells without buds. Cells in which buds were less than one-half the diameter of the mother cells were defined as cells with small buds. For each time point, at least 300 cells were scored microscopically for bud morphology. Thymidylate synthase activity per milliliter of synchronous culture was followed in whole cells permeabilized with Brij 58 by using the radiochemical assay described in the text. All the data presented are from a single synchronous culture. Cell samples for determining bud morphology were taken at 10-min intervals starting at t = 0 min, and samples for phosphatase activity, DNA content, and thymidylate synthase activity were harvested simultaneously at t = 0 and then at 10-min intervals after t = 13 min. The profile of thymidylate synthase activity versus time seen here was observed in each of three additional experiments (data not shown). Student's *t* test was used to compare activities at the peak from all four experiments, with the activities found at the first and the second time points immediately after the peak (i.e., a comparison of t = 53 min and t = 63 min for the above experiment). These paired comparisons showed that thymidylate synthase activity was significantly reduced at both the first and the second time points after the peak, with $P < 0.005$ and < 0.01, respectively.

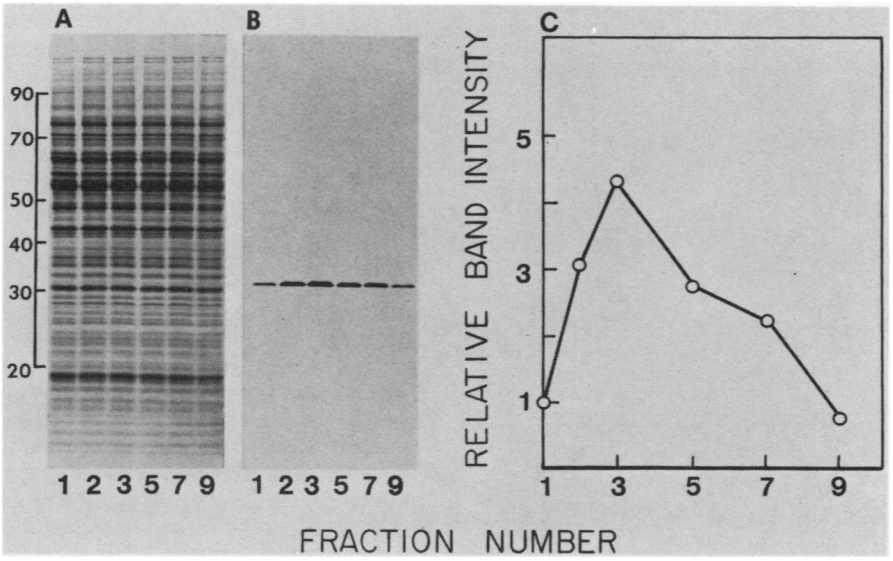

FIG. 3. FdUMP binding in elutriated cells. Samples of selected cell extracts used for determination of synthase activity (Fig. 1) were incubated with [^{3}H]FdUMP in the presence of N^5,N^{10}-methylenetetrahydrofolate for ternary complex formation, and the proteins were separated on a 12.5% sodium dodecyl sulfate-polyacrylamide gel. The Coomassie blue-stained gel is shown in (A), whereas (B) represents a fluorogram of the gel after 60 days of exposure at −80°C. A molecular weight scale (in thousands) is shown on the left. The molecular weight of the synthase subunit is estimated at 32,000, in close agreement with the estimate of 65,000 for the enzyme dimer (9). The relative band intensity of the ternary complex, measured by scanning a 14-day exposure, is shown in (C).

release assay or FdUMP binding, in whole cells or in cell extracts implies that the periodic pattern is real rather than an artifact of extraction method or assay technique. Furthermore, the cell cycle dependence of thymidylate synthase has been demonstrated in synchronous populations of *S. cerevisiae* cells derived by two independent methods and therefore is highly unlikely to have been induced by some incidental experimental manipulation.

The decrease in thymidylate synthase activity and FdUMP-binding capacity after S phase indicates that enzyme levels during synchronous growth may be determined in part by the instability of the enzyme. Bisson and Thorner (9) have demonstrated the extreme instability of the yeast synthase in cell extracts, and we have observed differential lability of the enzyme relative to other proteins both in vitro and in vivo (unpublished data). Synthase instability in vivo has also been reported in mammalian cell lines (47) and is likely to be a significant component of periodic thymidylate synthase expression in eucaryotes.

Relative levels of thymidylate synthase mRNA in synchronous cells. To investigate transcriptional involvement in the observed periodicity, we used the cloned yeast thymidylate synthase gene (*TMP1*) to determine the relative levels of *TMP1*-encoded RNA present during synchronous growth of AH22. Evidence verifying that the cloned DNA used as probe is indeed the *TMP1* structural gene is given in Fig. 5. Here it is shown that the thymidylate synthase expressed by the *TMP1* clone in *E. coli* is identical in size to the yeast enzyme and clearly different from *E. coli* synthase. These data corroborate experiments in which *TMP1* sequences were localized based on the ability of various subclones and BAL 31-generated deletions containing this region to complement *thyA* mutants of *E. coli* and *tmp1* mutants of yeast (data not shown). Furthermore, the nucleotide sequence of the gene (unpublished data) predicts an amino acid sequence which agrees with the published amino-terminal protein sequence (9).

Total RNA was isolated at 10-min intervals from synchronously growing *S. cerevisiae*, fractionated by glyoxal gel electrophoresis, and transferred to nylon membranes. *TMP1* mRNA was identified and relative levels were determined by hybridization to the radiolabeled *Hin*dIII to *Eco*RI fragment of the *TMP1* sequence (45). Autoradiographic analysis of the resulting Northern blot (Fig. 4c) shows that the *TMP1*-encoded transcript fluctuates in a periodic fashion during synchronous growth. Levels of *TMP1* mRNA began to increase immediately after α-factor release and peaked at 20 min as the cells entered S period. The amount of *TMP1* transcript fell after 20 min and reached basal levels by the end of S period. The roughly 10-fold-elevated *TMP1* mRNA maximum at 20 min coincided with the time in the cell cycle when thymidylate synthase levels were increasing at their maximum rate (Fig. 2 and 4). Synthase mRNA accumulated with a minimum doubling time of ca. 5 min at around the G1-S interface and then decayed with a minimum half-life of ca. 7 min during S period. The periodic accumulation and disappearance of the *TMP1* transcript shown in Fig. 4 is typical of that observed in three separate experiments with α-factor-synchronized AH22 (data not shown). We also monitored levels of the *URA3* transcript during synchronous growth of AH22. The *URA3* message did not fluctuate during the cell cycle but was present in constant amounts throughout the first synchronous cell division (Fig. 4). The noncyclic regulation of *URA3* is consistent with the observations of Elliot and McLaughlin (17) and Lörincz et al. (31), who observed, using two-dimensional gels, that extremely few proteins (ca. 1%) followed throughout the cell cycle of *S. cerevisiae* showed periodic expression.

DISCUSSION

Using synchronous populations of *S. cerevisiae* cells generated by two methods, we have shown that active thymidylate synthase, as measured by radiochemical assay as well as by FdUMP binding, accumulates during S period.

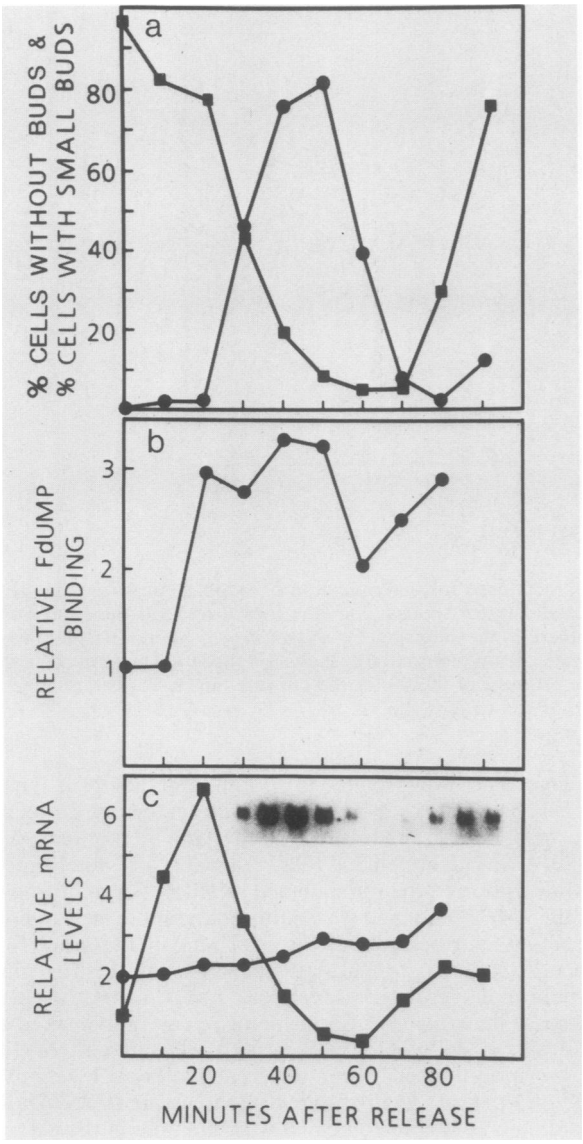

FIG. 4. Levels of FdUMP binding and *TMP1* transcript during synchronous growth after release from α-factor arrest. (a) Percentage of cells without buds (■) and with small buds (●) at different times after release. (b) Levels of FdUMP-binding activity quantitated as described in the legend to Fig. 3. The points represent an average derived from two separate fluorograms. (c) Quantitation of thymidylate synthase transcript. RNA was extracted from cells removed at the indicated times. The RNA was analyzed on a Northern gel, using the labeled *Eco*RI to *Hin*dIII fragment of the *S. cerevisiae TMP1* gene to identify the *TMP1* transcript. The autoradiogram is shown in the upper right-hand corner. Only a single RNA species was detected, 1.1 kb in length. Relative levels of *TMP1* mRNA at various times during synchronous growth were quantitated by densitometry (■). The hybridized DNA was eluted by washing the gel in 0.1× wash buffer (46) (1× wash buffer contains 50 mM Tris-hydrochloride [pH 8.0], 2 mM EDTA, 0.5% sodium pp_i, 0.02% bovine serum albumin, Ficoll, and polyvinyl pyrolidone) for 2 h at 65°C, and the blot was reprobed with the 0.9-kb *Pst*I to *Sma*I fragment of plasmid YIp5, which contains the yeast *URA3* gene (●). Whereas the data in (a) and (c) represent the same synchrony, the FdUMP binding data in (b) are from an independent experiment. The timing of the synchronies in these two cases, however, was indistinguishable.

These results are in basic agreement with the data obtained for several higher eucaryotic organisms, showing that thymidylate synthase activity is periodic during the cell cycle, with maximum activity occurring during S phase (2, 38, 42). With the cloned yeast synthase gene available, a detailed study of the processes regulating the periodicity of the enzyme is now possible. It has been previously shown that yeast histone expression is similarly cell cycle dependent (26, 31). The histones and thymidylate synthase are thus contrasted to the great majority of yeast proteins analyzed to date, which are continuously rather than periodically expressed (17, 31) as demonstrated here for *S. cerevisiae* invertase and acid phosphatase. This suggests that a small class of cellular proteins, typified by thymidylate synthase and histones, which are directly involved in chromatin metabolism, have their regulation coupled to DNA synthesis. An interesting question is whether other enzymes involved in providing DNA precursors, such as ribonucleotide reductase, are also periodically expressed in *S. cerevisiae*.

The data presented here indicate that the periodic increase in thymidylate synthase levels is determined primarily at the level of its transcript. Elevated levels of *TMP1*-encoded RNA are restricted to a small portion of the cell cycle; peaking at the beginning of S period. Whether the periodic accumulation of *TMP1* mRNA results from periodic synthe-

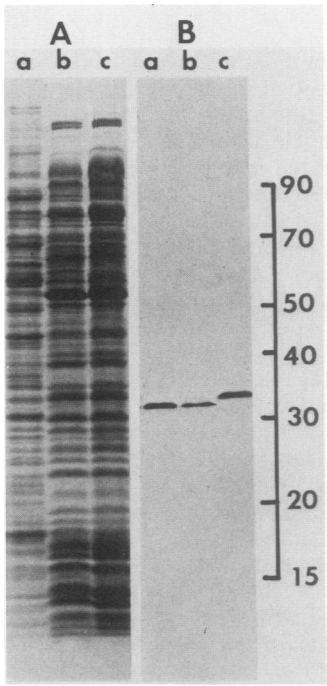

FIG. 5. Expression of yeast thymidylate synthase by the cloned *TMP1* gene in *E. coli*. (A) Sonic extracts were prepared from *S. cerevisiae* SKQ2N (lane a), from the *E. coli thyA* strain Rue10, which had been transformed by the *TMP1*-carrying plasmid pTL221 (lane b), or by the *thyA*-containing plasmid pBTAH (lane c). Ternary complex formation with [^{3}H]FdUMP and N^5,N^{10}-methylenetetrahydrofolate and electrophoresis were as described in the text and in the legend to Fig. 3, except that for lane c the specific activity of the FdUMP was diluted 80-fold. (B) The fluorogram shows the *TMP1*-encoded enzyme in *E. coli*, identical in size (32,000 daltons) to thymidylate synthase produced in *S. cerevisiae* (cf. lanes a and b) and migrating more rapidly than the bacterial synthase (cf. lanes b and c). Untransformed Rue10 forms no detectable ternary complex (5). The molecular weight scale to the right is in thousands.

sis or is the result of a constant rate of transcription with differential stability during the cell cycle is not known. We do, however, favor the hypothesis of periodic activation of transcription based on the following. (i) There is no evidence of breakdown products of the *TMP1* transcript, which argues against differential synthase mRNA stability through the cell cycle. (ii) In fusions placing β-galactosidase under control of the *TMP1* regulatory region, β-galactosidase is periodically expressed (manuscript in preparation). This suggests that periodicity may be controlled by a *TMP1* regulatory element 5′ to the gene rather than by an innate property of *TMP1* mRNA which renders it susceptible to breakdown in a cell cycle-dependent manner. Furthermore, it appears that the *TMP1* transcript is not synthesized as a precursor, as no precursor species were detected. In addition, it appears that the transcript is utilized immediately and that mRNA levels correlate with the rate of accumulation of thymidylate synthase. This is evidenced by the mRNA peak coinciding with the maximal rate of thymidylate synthase synthesis at the beginning of S phase. Also, there is no net increase in synthase levels when the transcript is present in basal amounts before and after the mRNA peaks. Transcript levels fall dramatically after the beginning of S phase, with a half-life of ca. 7 min. This rapid disappearance of the thymidylate synthase transcript presumably results in the immediate cessation of enzyme synthesis.

In addition to control of thymidylate synthase levels by precise regulation of its transcript, synthase levels may also be regulated at the level of enzyme stability. This is suggested by a drop in both enzyme activity and FdUMP binding after the end of S phase. This combination of the periodic appearance of the *TMP1* transcript and protein instability would enable *S. cerevisiae* to modulate precisely its synthase levels over times as short as a single cell cycle. Cell division cycle (*cdc*) mutants (23, 24), which block *S. cerevisiae* at different stages of the cell cycle, as well as the use of inhibitors of DNA, RNA, and protein synthesis will be useful in analyzing the stability of both the enzyme protein and the synthase transcript at different times during the cycle.

Thymidylate synthase is the sole source of dTMP for the replication machinery of the yeast cell. Not only does *S. cerevisiae* lack thymidine kinase (22), but yeast cells are ordinarily impermeable to thymine derivatives, including dTMP (11). As a result, thymidylate synthase mutants starved for dTMP arrest in S phase, with a morphological phenotype characteristic of DNA elongation mutants (7, 24). One of the original *cdc* mutants, *cdc21* (24), has in fact been shown to be at the *TMP1* locus (7). Despite the vital role of the synthase in progression through the cell cycle, its specific activity in *S. cerevisiae* cells is extremely low (7; this study). This precarious situation is presumably relieved by the boost in enzyme levels in S phase, facilitating completion of DNA synthesis.

The presence of the cell cycle control region in the *TMP1* clones as well as the thymidylate synthase-β-galactosidase fusions (unpublished data) should aid in decoding cell cycle signals and in delineating those features of the gene which respond to these signals. The regulatory sequence responsible for periodic expression of histone 2A in yeasts has been previously shown to be coincident with a region responsible for autonomous replication (39). Whether similar sequences regulate *TMP1* periodicity is not yet known, and exactly how sufficient synthase levels are ensured to allow the successful completion of the replication cycle remains the subject of further investigation.

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