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Control of Thymidylate Synthase mRNA Content and Gene Transcription in an Overproducing Mouse Cell Line

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We studied the content and metabolism of thymidylate synthase mRNA in cultured mouse fibroblasts that were undergoing a serum-induced transition from the resting to growing state. The studies were performed with a 5-fluorodeoxyuridine-resistant 3T6 cell line (LU3-7) that overproduces the enzyme and its mRNA about 50-fold and that regulates the expression of the thymidylate synthase gene in the same manner as the parental cell line. We have previously shown that the rate of synthesis of thymidylate synthase increases at least ninefold when the serum-stimulated cells traverse the S phase. Here we show, by Northern blot analysis, that thymidylate synthase mRNA increased 20- to 40-fold as cells progressed from resting to late S phase. About 85% of poly(A)$^+$ thymidylate synthase mRNA was associated with polysomes at all times. The increase in thymidylate synthase poly(A)$^+$ mRNA content was the result of an eightfold increase in the rate of production of this species, as shown by pulse-labeling studies. Pulse-chase analysis revealed that the half-life of thymidylate synthase poly(A)$^+$ mRNA was similar in resting (9 h) and growing (7 h) cells. The rate of transcription of the thymidylate synthase gene, as determined in isolated nuclei, increased only by a factor of three to four during the S phase. Since the content of the message increased to a much greater extent than the rate of transcription of the gene, posttranscriptional controls must also play a role in regulating the content of thymidylate synthase mRNA under these conditions. Our results suggest that the cell may regulate the distribution of thymidylate synthase mRNA between a relatively stable poly(A)$^+$ RNA species and a labile poly(A)$^-$ RNA species.

Thymidylate synthase (TS; EC 2.1.1.45) is the enzyme that catalyzes the reductive methylation of dUMP during the de novo synthesis of TMP. Because TS is an essential enzyme in proliferating cells, it is an important target enzyme in cancer chemotherapy (6). Many laboratories have shown that in mammalian as well as in yeast cells, the level and enzymatic activity of TS are high when cells are engaged in DNA replication but very low at other times in the cell cycle or in nondividing cells (5, 13, 17, 22, 27). We have shown that when resting (G0) mouse 3T6 fibroblasts are stimulated with serum to reenter the cell cycle, the amount of TS increases about 10-fold between 10 and 25 h following serum addition (21). Although the increase in TS activity is closely coordinated in time with DNA replication, it is not blocked by the presence of inhibitors of DNA synthesis. This shows that these two events are not directly coupled.

To facilitate a more direct analysis of TS gene expression, we isolated a 5-fluorodeoxyuridine (FdUrd)-resistant 3T6 cell line (LU3-7) that overproduces TS by a factor of about 50 (23). The overproduced TS appears to be the same as the normal enzyme, as determined by several different criteria. Recently, we cloned DNA corresponding to nearly the entire length of TS mRNA into pBR322 (7). Using this plasmid as a hybridization probe, we showed that TS mRNA is overproduced to the same extent as the enzyme in LU3-7 cells (7). Southern blot analysis showed that the overproduction of TS and its mRNA was due to a corresponding increase in the number of copies of the TS gene (11). TS gene expression appears to be regulated in the same manner in the LU3-7 cells as in the parental 3T6 cells (12). Therefore, the LU3-7 cell line should be a convenient model system for studying TS gene expression.

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In this report we show that when serum-arrested LU3-7 cells are stimulated to reenter the cell cycle, the increase in the rate of synthesis of TS is the result of an increase in TS mRNA content. This is due in part to an increase in TS gene transcription and in part to regulation at the posttranscriptional level.

MATERIALS AND METHODS

Cell culture. LU3-7 cells are a FdUrd-resistant derivative of mouse 3T6 fibroblasts (23). They were maintained on plastic petri dishes (Becton Dickinson Labware) in the Dulbecco-Vogt modification of Eagle's medium (GIBCO Laboratories) supplemented with 10% calf serum (Colorado Serum), 3 μM FdUrd, 1 mM uridine, and 1 mM cytidine. Resting LU3-7 cells were prepared by seeding 7×10^4 cells per cm^2 in medium containing 0.5% serum but lacking FdUrd, uridine, and cytidine. The medium was replaced on the second and fourth days, and the cells were used for an experiment on the seventh day following seeding (14). Resting cells were stimulated to reenter the cell cycle by feeding them fresh medium containing 10% calf serum.

Isolation and analysis of cytoplasmic RNA. Cultures of cells were harvested and lysed with Nonidet P-40, and the cytoplasmic compartment was isolated (14). Polysomes were fractionated as described by Geyer et al. (8). Extracts were digested with pronase, and RNA was purified by phenol-chloroform extraction at room temperature (14). Poly(A)$^+$ RNA was isolated by chromatography on an oligo(dT) cellulose column (2). The amount of poly(A)$^+$ mRNA in the RNA preparation was determined by the [^{3}H]poly(U) binding assay described previously (3).

Northern blot analysis. Northern blot analysis was performed essentially as described by Lehrach et al. (15) and Thomas (28). Briefly, RNA was denatured and electrophoresed on 1.5% agarose gels containing formaldehyde. The RNA was fragmented by brief exposure to alkali and trans-

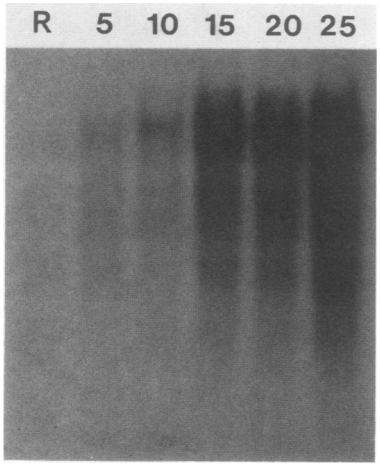

FIG. 1. TS mRNA content in serum-stimulated LU3-7 cells. Cultures of resting (R) cells were serum stimulated at time zero. At the indicated times (in hours), cells were harvested, and total cytoplasmic RNA was isolated. Samples containing equal amounts of poly(A) [as determined by the [^{3}H]poly(U) binding assay] were denatured and electrophoresed on 1.5% agarose gels in the presence of formaldehyde. RNA was transferred to nitrocellulose and probed with nick-translated pMTS-3. The most intense band at the top of the autoradiogram represents the major 1.3-kb TS mRNA species.

ferred to nitrocellulose. The filter was prehybridized and hybridized as described previously (11) and was washed extensively with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 65°C prior to autoradiography. The probe was the plasmid pMTS-3 (7), which contains a 1-kilobase (kb) insert corresponding to nearly the entire length of mouse TS mRNA. The plasmid was labeled by nick translation (18) to a specific activity of 10^8 cpm/μg.

DNA-excess filter hybridization. Five micrograms of pMTS-3 was denatured in alkali and immobilized on a 13-mm nitrocellulose filter (10). Control filters were treated in a similar manner except that DNA was omitted. All filters were prehybridized for at least 4 h (24) to reduce nonspecific binding of labeled RNA to the filters. Hybridization, washing, and incubation of the filters with RNase was conducted as described by Hendrickson et al. (10). Radioactivity associated with the filters was determined in a scintillation counter.

In vitro transcription. Nuclei were prepared and transcribed in vitro at 25°C by the procedure of Santiago et al. (24). The labeled substrate was [^{3}H]UTP (40 Ci/mmol; ICN Pharmaceuticals Inc.). Heparin (150 μg/ml) was included in the reaction to inhibit nucleases and to prevent reinitiation of transcription. In earlier studies of dihydrofolate reductase gene transcription, we showed that incubations ranging from 20 to 60 min gave essentially the same results. Therefore, to maximize the amount of incorporation, we used a 60-min incubation period in the present transcription analyses. RNA was purified by phenol-chloroform extraction followed by exclusion chromatography on Sephadex G100 (to eliminate low-molecular-weight contaminants). The amount of labeled RNA corresponding to TS mRNA was determined by a filter hybridization procedure.

RESULTS

TS mRNA content in serum-stimulated cells. Results of our previous studies have shown that when resting LU3-7 cells are stimulated to proliferate, the rate of DNA synthesis and the percentage of cells engaged in DNA synthesis remain at the resting level for the first 10 h and then increase sharply as the cells begin DNA replication. The rate of synthesis of the TS enzyme remains constant until the cells begin DNA replication and then increases at least ninefold between 10 and 25 h following stimulation (12). Here we examined TS mRNA content and metabolism in stimulated cells to determine if these increased in a similar manner. Total cytoplasmic RNA was prepared from cells stimulated for various times and electrophoresed on a denaturing agarose gel. The amount of RNA analyzed was adjusted so that each RNA sample contained the same amount of poly(A)$^+$ mRNA [as determined by [^{3}H]poly(U) hybridization]. RNA was transferred to nitrocellulose and probed with nick-translated pMTS-3. Figure 1 shows that the TS mRNA content remained at low levels until the cells entered the S phase and then increased dramatically at later times.

The extent of the increase was determined by dilution analysis of poly(A)$^+$ mRNA isolated from cells stimulated for 5 and 25 h. Figure 2 shows that the TS mRNA content increased 20- to 40-fold during this interval. This result agrees reasonably well with the increase in the rate of synthesis of the enzyme. When these analyses were performed with total cytoplasmic RNA instead of poly(A)$^+$ RNA, nearly identical results were obtained (data not shown). The great majority of the TS mRNA had a size of about 1.3 kb, although several species with higher molecular sizes were also observed. The relative amount and the sizes

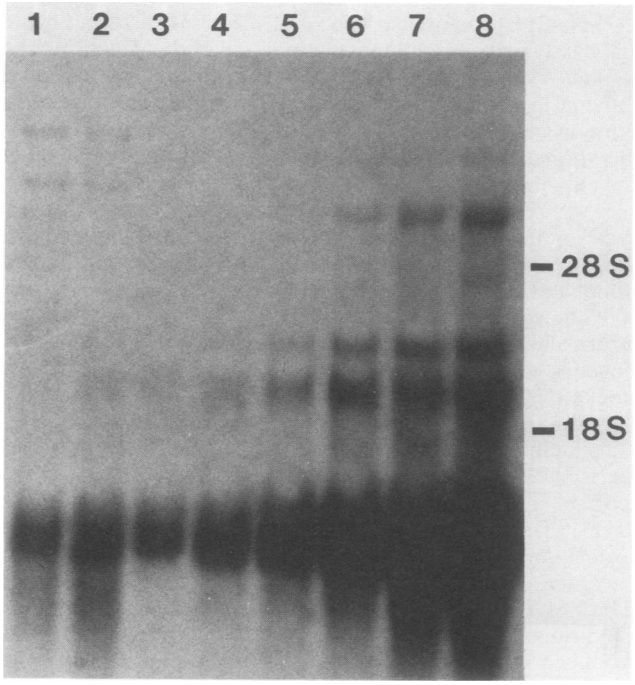

FIG. 2. Increase in TS mRNA as determined by dilution analysis. Poly(A)$^+$ RNA was isolated from LU3-7 cells that were serum stimulated for 5 or 25 h. The dilutions indicated below were electrophoresed and analyzed as described in the legend to Fig. 1. The intensities of the signals were compared to estimate the fold increase in TS mRNA content. Lanes 1 and 2 are two different preparations of RNA from cultures stimulated for 5 h, and lanes 3 through 8 contain RNA from a culture stimulated for 25 h. Lanes 1, 2, and 8 contain the same amount of poly(A)$^+$ RNA, as determined by the [^{3}H]poly(U) binding assay; the other lanes contain RNA diluted as indicated: lane 3, 1/40; lane 4, 1/20; lane 5, 1/10; lane 6, 1/5; lane 7, 1/2. Size markers are 28S and 18S rRNA.

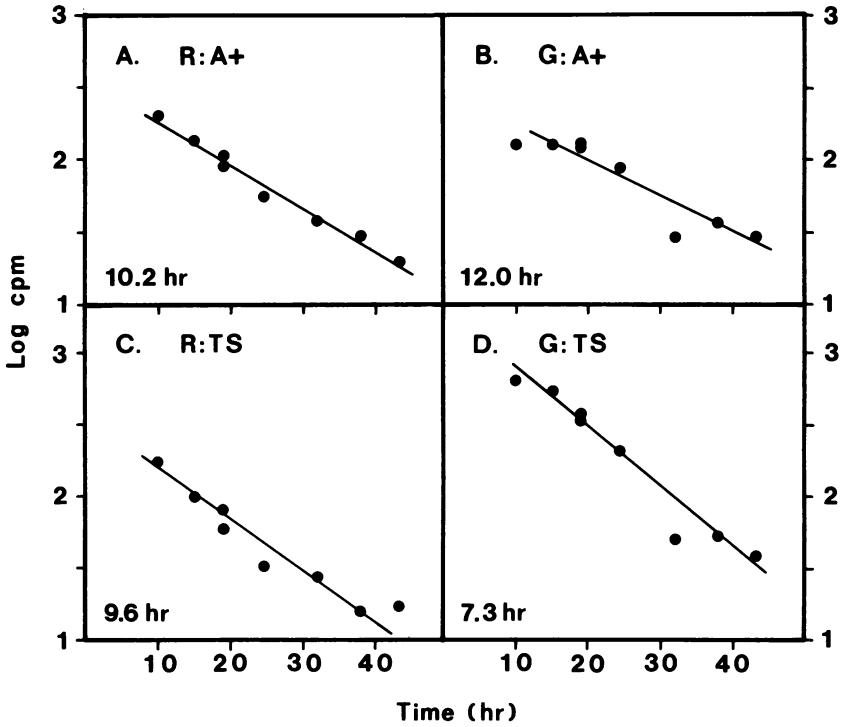

FIG. 3. Stability of TS mRNA. Cultures of exponentially growing (G) or resting (R) LU3-7 cells were labeled with [^{3}H]uridine for 5 h. At time zero, the medium was replaced with medium lacking isotope and containing 5 mM uridine, 2.5 mM cytidine, and serum at the appropriate level. At the indicated times, cultures were harvested and the amount of radioactivity in total poly(A)$^+$ mRNA (A$^+$) and TS poly(A)$^+$ mRNA (TS) were determined. These values were plotted on a logarithmic scale as a function of chase time. The values for total poly(A)$^+$ mRNA represent 0.1% of the total sample. The curve was fit by least-squares analysis, and the half-lives are shown at the bottom left of each panel.

of some of the larger species appeared to be different at 5 and 25 h following stimulation. The structure and significance of these larger species remain to be determined.

Synthesis and stability of TS mRNA. The increase in TS mRNA content could be the result of an increase in the rate of production of TS mRNA or the stabilization of the message. Since TS mRNA was overproduced about 50-fold in LU3-7 cells (7), it was present at sufficient levels to permit direct examination of these parameters by DNA-excess filter hybridization. The half-life of poly(A)$^+$ TS mRNA was measured in resting and exponentially growing cells. Cultures were labeled with [^{3}H]uridine for 5 h and then chased with an excess of unlabeled uridine and cytidine. Figure 3 shows that the half-life of total poly(A)$^+$ mRNA was about 10 h in resting LU3-7 cells and 12 h in growing cells. These half-lives were slightly longer than those observed for 3T6 cells (1). The half-life of TS poly(A)$^+$ mRNA was about 9.6 h in resting and 7.3 h in growing LU3-7 cells. Since poly(A)$^+$ TS mRNA did not appear to be any more stable in cycling versus noncycling cells, the increase in content of this species must be the result of an increase in the rate of production.

The rate of production of cytoplasmic TS mRNA was studied by measuring the incorporation of [^{3}H]uridine into poly(A)$^+$ TS mRNA relative to total mRNA during a 2 h labeling period at various times following serum stimulation. Figure 4A shows that the rate of labeling began to increase between 4 and 8 h following serum addition and reached the maximum value by 18 h. The rate increased by a factor of eight when cells stimulated for 1 and 18 h were compared.

A different result was obtained when we analyzed poly(A)$^-$ TS RNA. This RNA is defined operationally as RNA that did not bind to the oligo(dT) cellulose column, although it may still retain a short oligo(A) tail. This species is referred to as RNA rather than mRNA since much of it does not appear to be translationally active (see below). Figure 4B shows that the rate of labeling of this species [relative to total cytoplasmic poly(A)$^+$ RNA] increased less than twofold as cells traversed the S phase. We were surprised to see that about 70% of the pulse-labeled cytoplasmic TS RNA was in the poly(A)$^-$ fraction prior to 5 h of stimulation, whereas about 30% was in the poly(A)$^-$ fraction between 18 and 25 h following stimulation. The rate of labeling of total [poly(A)$^+$ and poly(A)$^-$] cytoplasmic TS RNA relative to total poly(A)$^+$ mRNA increased by a factor of three to four by 18 h following stimulation.

The labeling kinetics of cytoplasmic TS RNA were examined in more detail for pre-S phase and S phase cells. Cultures were stimulated for either 1 or 18 h and then labeled for intervals ranging from 1 to 4 h. We found that for all labeling intervals examined, 65 to 75% of the TS RNA was in the poly(A)$^-$ fraction for the cells stimulated for 1 h, whereas 30 to 40% was in the poly(A)$^-$ fraction for the cells stimulated for 18 h (data not shown).

Polysome distribution of TS mRNA. It was possible that the poly(A)$^-$ TS RNA did not represent functional TS mRNA but rather degradation products of poly(A)$^+$ TS mRNA (or nuclear TS RNA species). If so, the poly(A)$^-$ TS RNA would be expected to be smaller than intact TS mRNA, heterogeneous in size, and not associated with ribosomes. The polysome distribution of TS mRNA was determined by stimulating cultures of cells for 1 or 18 h and then labeling them for 2 h with [^{3}H]uridine. Cytoplasmic compartments were separated into polysomes (>100S) and smaller particles

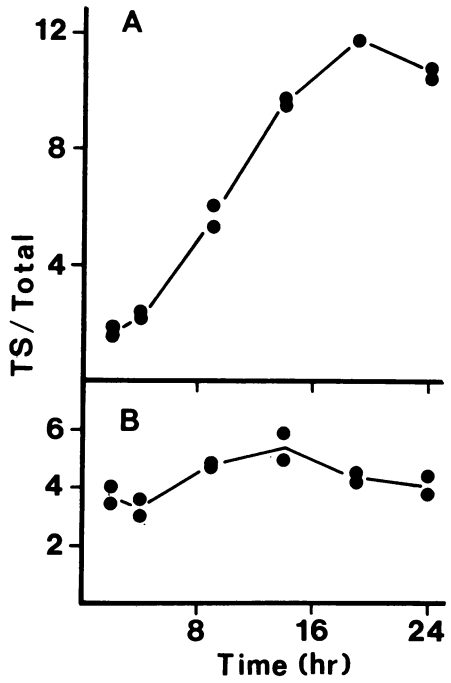

FIG. 4. Rate of production of TS mRNA in serum-stimulated cells. Cultures of resting LU3-7 cells were serum stimulated at time zero. At various times, cultures were labeled for 2 h with [^{3}H]uridine (125 μCi/ml) in medium containing 10% serum. After the labeling period the cultures were harvested, and poly(A)$^+$ and poly(A)$^-$ RNAs were isolated. The amount of labeled TS RNA in each fraction was determined by DNA-excess filter hybridization and normalized to the amount of radioactivity in total poly(A)$^+$ mRNA to give the relative rate of synthesis of TS poly(A)$^+$ (A) and TS poly(A)$^-$ (B) RNA. The values were multiplied by 10^4 and plotted at the midpoint of the labeling intervals. The result of two independent experiments are shown.

(<100S) by sucrose gradient sedimentation. Poly(A)$^+$ and poly(A)$^-$ RNAs were purified from both fractions, and the amount of labeled TS RNA in each was determined by filter hybridization. Table 1 shows that about 85% of the pulse-labeled poly(A)$^+$ TS mRNA was associated with the polysome fraction. In contrast, only about half of the pulse-labeled poly(A)$^-$ TS RNA was associated with ribosomes.

TABLE 1. Distribution of TS mRNA in subpolysomal and polysomal fractions[a]

Time of stimulation and fraction	Percentage of labeled RNA in the following:		
	Poly(A)$^+$ TS RNA	Poly(A)$^-$ TS RNA	Total poly(A)$^+$ RNA
1 h			
Subpolysomal	12	49	27
Polysomal	88	51	73
18 h			
Subpolysomal	15	40	34
Polysomal	85	60	66

[a]Cultures of resting LU3-7 cells were serum stimulated for 1 or 18 h and then labeled for 2 h with [^{3}H]uridine (125 μCi/ml). Cytoplasmic extracts were separated into subpolysomal and polysomal fractions. Each fraction was then separated into poly(A)$^+$ and poly(A)$^-$ RNA, and the amount of labeled TS RNA in each sample was determined and expressed as a percentage.

To determine the size of the TS RNA in each fraction, unlabeled cytoplasmic extracts were isolated from cultures stimulated for 2 and 19 h and separated into polysomal and subpolysomal fractions as described above. Purified RNA samples were subjected to Northern blot analysis (Fig. 1). Figure 5 shows that the polysomal and subpolysomal poly(A)$^+$ TS mRNA migrated as one major species (about 1.3 kb) and several larger, minor species. Poly(A)$^-$ TS RNA was generally much smaller than poly(A)$^+$ TS mRNA (even though some faint high-molecular-weight species were visible) and had a heterogeneous size distribution. Although some of the size reduction is due to the loss of the poly(A) segment, our results are consistent with the possibility that at least a portion of this species represents a breakdown product. We were surprised that the polysomal poly(A)$^-$ TS RNA was also small. If this RNA is being translated, the translation products of the smallest species must represent fragments of the TS protein since the 1.3-kb mRNA is just barely large enough to code for TS, which has a molecular weight of about 38,000 (23). It is unlikely that the degradation occurs during RNA purification since we would expect the poly(A)$^+$ TS mRNA to be degraded as well.

Figure 5 also shows that in G0-G1 phase cells, most of the cytoplasmic TS RNA mass is in the poly(A)$^+$ RNA fraction in spite of the fact that only about 30% of the pulse-labeled cytoplasmic TS RNA is poly(A)$^+$. This suggests that at least a portion of the cytoplasmic poly(A)$^-$ TS RNA is much less stable than poly(A)$^+$ TS mRNA.

TS gene transcription. To determine what is responsible

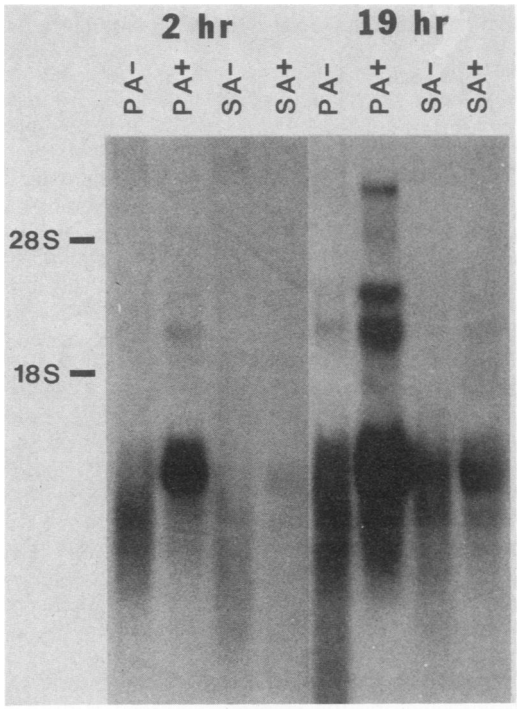

FIG. 5. Northern blot analysis of TS RNA in the polysomal and subpolysomal fractions. Cytoplasmic extracts prepared from cultures that were serum stimulated for 2 h (lanes 1 through 4) and 19 h (lanes 5 through 8) were separated into subpolysomal (S) and polysomal (P) fractions on linear 15 to 40% sucrose gradients. RNA from each sample was purified and separated into poly(A)$^+$ RNA (A$^+$) and poly(A)$^-$ RNA (A$^-$) fractions. The entire RNA preparation was electrophoresed and analyzed as described in the legend to Fig. 1. Size markers were 28S and 18S rRNA.

for the increase in TS mRNA production following serum stimulation, we measured the rate of transcription of the TS gene. Nuclei were isolated from cultures at various times after stimulation, and the rate of incorporation of [^{3}H]UTP into TS heterogeneous nuclear RNA (hnRNA) relative to total hnRNA was determined. Figure 6 shows that the rate of TS gene transcription increased at the same time as the increase in labeling of cytoplasmic TS mRNA. The magnitude of the increase (three- to fourfold) was only about half of that observed for TS poly(A)$^+$ mRNA, but was similar to that observed for total cytoplasmic TS mRNA. This is consistent with the idea that the production of total cytoplasmic TS RNA is regulated by control of the transcription of the TS gene rather than by control of the efficiency of conversion of TS hnRNA into cytoplasmic TS RNA. However, we cannot rule out the possibility that some potential TS mRNA sequences are degraded in the nucleus.

When cells were serum stimulated in the presence of 5 μg of cytosine arabinoside per ml, which inhibits DNA synthesis by greater than 95%, the increase in TS gene transcription was not affected. This shows that there is no direct coupling between TS gene transcription and DNA replication. In fact, the increase is somewhat greater in the drug-treated cells than in control cells. This is probably due to the fact that when cells are stimulated in the presence of cytosine arabinoside, the increase in the rate of synthesis of total hnRNA (that normally occurs during the S phase) is prevented (19). Therefore, the rate of synthesis of TS hnRNA relative to total hnRNA is greater in cells treated with cytosine arabinoside than in control cells.

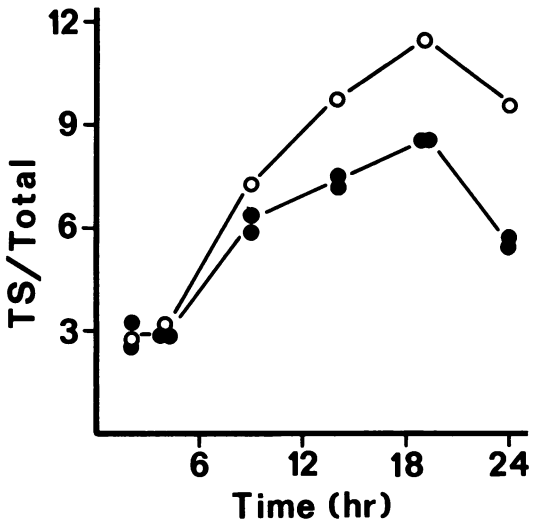

FIG. 6. TS gene transcription in serum-stimulated cultures. Cultures of resting LU3-7 cells were serum stimulated at time zero in the presence (○) or absence (●) of 5 μg of cytosine arabinoside per ml. At the indicated times, cultures were harvested and nuclei prepared. The nuclei were transcribed in vitro, and the rate of synthesis of TS RNA relative to total RNA was determined. These values were multiplied by 10^5 and plotted versus time.

DISCUSSION

The isolation of a TS-overproducing cell line that regulates TS gene expression normally and the cloning of mouse TS cDNA have permitted, for the first time, a direct analysis of the metabolism of TS mRNA and the transcription of the TS gene in eucaryotic cells. Such studies are not possible with normal cells since TS mRNA represents such a small fraction of total cellular mRNA. It is clear that the increase in the rate of synthesis of TS that occurs as serum-stimulated cells traverse the S phase is due to an increase in TS mRNA content. Since the majority of TS mRNA is associated with polysomes at all times, and since there is a good correspondence between the increase in TS mRNA content and the rate of synthesis of the enzyme, it is unlikely that TS gene expression is regulated to a significant extent by control of the translation of TS mRNA. The increase in poly(A)$^+$ TS mRNA content is the result of an increase in the rate of production rather than the stabilization of this species. The increase in TS mRNA production is due in part to an increase in the rate of transcription of the TS gene. However, since the TS mRNA content increases to a greater extent than the rate of transcription of the gene, posttranscriptional controls must also play a role in regulating TS gene expression.

Several observations suggest that regulation of the polyadenylation state of cytoplasmic TS RNA may be important in the control of TS gene expression. We found that in pulse-labeled cells, about 70% of labeled TS cytoplasmic RNA was in the poly(A)$^-$ RNA fraction in G0-G1 phase cells. This decreased to about 30% in S phase cells. Furthermore, it appears that at least a portion of the cytoplasmic poly(A)$^-$ TS RNA is less stable than poly(A)$^+$ TS mRNA. This is in line with results of previous studies which showed that cytoplasmic messages that lack poly(A) turn over more rapidly than those that are poly(A)$^+$ (29). Thus, the cell may regulate the TS mRNA content in part by controlling the rate of transcription of the TS gene and in part by controlling the distribution of cytoplasmic TS RNA between a relatively stable poly(A)$^+$ TS RNA species and a labile poly(A)$^-$ TS RNA species. Increased production of cytoplasmic poly(A)$^-$ TS RNA may be the result of increased transport of spliced, poly(A)$^-$ TS hnRNA from the nucleus to the cytoplasm or from an increase in the rate of deadenylation of cytoplasmic poly(A)$^+$ TS mRNA. Of course, other explanations for our observations are also possible. For example, during cell fractionation, nuclear poly(A)$^-$ TS RNA processing intermediates or breakdown products may leak into the cytoplasmic compartment more readily in G0-G1 phase cells than in S phase cells.

Northern blot analysis revealed several high-molecular-weight cytoplasmic TS RNA species in both poly(A)$^+$ and poly(A)$^-$ RNA fractions. These species were also observed in the parental 3T6 cells (7). The larger species may represent processing intermediates that have leaked from the nucleus. Another possibility is that they represent translatable TS mRNAs that differ (perhaps) in the length of the 3′ untranslated region of the mRNA, as observed previously for dihydrofolate reductase mRNA (25). The fact that the larger species were present in polysomal RNA preparations is consistent with the latter possibility.

The mechanisms for regulating several other genes coding for S phase proteins have been studied in some detail. TS gene regulation is similar to these in some respects and distinctly different in others. For example, histone gene expression is regulated in part at the transcriptional level and in part by control of the stability of histone mRNA (26). However, histone gene expression is directly coupled to DNA replication (26), whereas TS gene expression is not. Dihydrofolate reductase gene expression can be regulated at either the transcriptional or posttranscriptional level, depending on the method of cell synchrony or the physiological state of the cell (13). Stationary phase (16) or glutamine-starved cells (4) have a lower efficiency of rate of

dihydrofolate reductase hnRNA processing, respectively, than do rapidly growing cells. However, cells synchronized by release from serum limitation, as in this study, regulate dihydrofolate reductase gene expression at the transcriptional level (24). The cellular thymidine kinase gene appears to be regulated at the posttranscriptional level (9), and sequences internal to the thymidine kinase gene appear to be important for controlling the level of this mRNA (20). It will be interesting to see if the cell regulates this gene by regulating the processing or the stability of this mRNA. Thus, from these few examples, it appears that there is no universal mechanism for regulating the expression of all genes coding for S phase proteins.

It is still possible that several genes may be controlled by the same regulatory factor, such as a transcriptional control protein. These common factors would be expected to bind to similar oligonucleotides, probably in the vicinity of the promoter region. Therefore, it is important to compare the sequences upstream of the transcriptional start site for genes whose transcription increases in a coordinated manner, such as the genes for dihydrofolate reductase and TS.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Abelson, H. T., L. F. Johnson, S. Penman, and H. Green.** 1974. Changes in RNA in relation to growth of the fibroblast. II. The lifetime of mRNA, rRNA and tRNA in resting and growing cells. Cell **1**:161–165.
2. **Aviv, H., and P. Leder.** 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid cellulose. Proc. Natl. Acad. Sci. USA **69**:1408–1412.
3. **Bishop, J. O., M. Rosbach, and D. Evans.** 1974. Polynucleotide sequences in eucaryotic DNA and RNA that form ribonuclease resistant complexes with polyuridylic acid. J. Mol. Biol. **85**:75–86.
4. **Collins, M. L., J.-S. R. Wu, C. L. Santiago, S. L. Hendrickson, and L. F. Johnson.** 1983. Delayed processing of dihydrofolate reductase heterogeneous nuclear RNA in amino acid-starved mouse fibroblasts. Mol. Cell. Biol. **3**:1792–1802.
5. **Conrad, A. H., and F. H. Ruddle.** 1972. Regulation of thymidylate synthetase activity in cultured mammalian cells. J. Cell Sci. **10**:471–486.
6. **Danenberg, P. V.** 1977. Thymidylate synthetase—a target enzyme in cancer chemotherapy. Biochim. Biophys. Acta **473**:73–92.
7. **Geyer, P. K., and L. F. Johnson.** 1984. Molecular cloning of DNA sequences complementary to mouse thymidylate synthase messenger RNA. J. Biol. Chem. **259**:7206–7211.
8. **Geyer, P. K., O. Meyuhas, R. P. Perry, and L. F. Johnson.** 1982. Regulation of ribosomal protein mRNA content and translation in growth-stimulated mouse fibroblasts. Mol. Cell. Biol. **2**:685–693.
9. **Groudine, M., and C. Casimir.** 1984. Post-transcriptional regulation of the chicken thymidine kinase gene. Nucleic Acids Res. **12**:1427–1446.
10. **Hendrickson, S. L., J.-S. R. Wu, and L. F. Johnson.** 1980. Cell cycle regulation of dihydrofolate reductase mRNA metabolism in mouse fibroblasts. Proc. Natl. Acad. Sci. USA **77**:5140–5144.
11. **Jenh, C.-H., P. K. Geyer, F. Baskin, and L. F. Johnson.** 1985. Thymidylate synthase gene amplification in fluorodeoxyuridine-resistant mouse cell lines. Mol. Pharmacol. **28**:80–85.
12. **Jenh, C.-H., L. G. Rao, and L. F. Johnson.** 1985. Regulation of thymidylate synthase enzyme synthesis in 5-fluorodeoxyuridine-resistant mouse fibroblasts during the transition from the resting to growing state. J. Cell. Physiol. **122**:149–154.
13. **Johnson, L. F.** 1984. Expression of dihydrofolate reductase and thymidylate synthase genes in mammalian cells, p. 25–47. *In* G. S. Stein and J. L. Stein (ed.), Recombinant DNA and cell proliferation. Academic Press. Inc., New York.
14. **Johnson, L. F., H. T. Abelson, H. Green, and S. Penman.** 1974. Changes in RNA in relation to growth of the fibroblast. I. Amounts of mRNA, rRNA and tRNA in resting and growing cells. Cell **1**:95–100.
15. **Lehrach, H., D. Diamond, J.M. Wozney, and H. Boedtker.** 1977. RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. Biochemistry **16**:4743–4751.
16. **Leys, E. J., G. F. Crouse, and R. E. Kellems.** 1984. Dihydrofolate reductase gene expression in cultured mouse cells is regulated by transcript stabilization in the nucleus. J. Cell Biol. **99**:180–187.
17. **Maley, F., and G. F. Maley.** 1960. Nucleotide interconversions. II. Elevation of deoxycytidylate deaminase and thymidylate synthetase in regenerating rat liver. J. Biol. Chem. **235**:2968–2970.
18. **Maniatis, T., A. Jeffrey, and D. G. Kleid.** 1975. Nucleotide sequence of the rightward operator of phage lambda. Proc. Natl. Acad. Sci. USA **72**:1184–1188.
19. **Mauck, J. C., and H. Green.** 1973. Regulation of RNA synthesis in fibroblasts during transition from resting to growing state. Proc. Natl. Acad. Sci. USA **70**:2819–2822.
20. **Merrill, G. F., S. D. Hauschka, and S. L. McKnight.** 1984. TK enzyme expression in differentiating muscle cells is regulated through an internal segment of the cellular tk gene. Mol. Cell. Biol. **4**:1777–1784.
21. **Navalgund, L. G., C. Rossana, A. J. Muench, and L. F. Johnson.** 1980. Cell cycle regulation of thymidylate synthase gene expression in cultured mouse fibroblasts. J. Biol. Chem. **255**:7386–7390.
22. **Rode, W., K. J. Scanlon, B. A. Moroson, and J. R. Bertino.** 1980. Regulation of thymidylate synthetase in mouse leukemia cells. J. Biol. Chem. **255**:1305–1311.
23. **Rossana, C., L. G. Rao, and L. F. Johnson.** 1982. Thymidylate synthetase overproduction in 5-fluorodeoxyuridine-resistant mouse fibroblasts. Mol. Cell. Biol. **2**:1118–1125.
24. **Santiago, C., M. Collins, and L. F. Johnson.** 1984. In vitro and in vivo analysis of the control of dihydrofolate reductase gene transcription in serum-stimulated mouse fibroblasts. J. Cell. Physiol. **118**:79–86.
25. **Setzer, D. R., M. McGrogan, J. H. Nunberg, and R. T. Schimke.** 1980. Size heterogeneity in the 3′ end of dihydrofolate reductase messenger RNAs in mouse cells. Cell **22**:361–370.
26. **Stein, G. S., J. L. Stein, and W. F. Marzluff.** 1984. Histone genes: structure, organization and regulation. John Wiley and Sons, Inc., New York.
27. **Storms, R. K., R. W. Ord, M. T. Greenwood, B. Mirdamadi, F. K. Chu, and M. Belfort.** 1984. Cell cycle-dependent expression of thymidylate synthase in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:2858–2864.
28. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
29. **Zeevi, M., J. R. Nevins, and J. E. Darnell, Jr.** 1982. Newly formed mRNA lacking polyadenylic acid enters the cytoplasm and the polyribosomes but has a shorter half-life in the absence of polyadenylic acid. Mol. Cell. Biol. **2**:517–525.