Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 8977–8981, October 1991
Cell Biology

Autoregulation of human thymidylate synthase messenger RNA translation by thymidylate synthase

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Communicated by J. Edward Rall, July 8, 1991 (received for review April 19, 1991)

ABSTRACT **Thymidylate synthase (TS; 5,10-methylenetetrahydrofolate:dUMP *C*-methyltransferase, EC 2.1.1.45) is essential for the *de novo* synthesis of thymidylate, a precursor of DNA. Previous studies have shown that the cellular level of this protein is regulated at both the transcriptional and post-transcriptional levels. The regulation of human TS mRNA translation was studied *in vitro* with a rabbit reticulocyte lysate system. The addition of purified human recombinant TS protein to *in vitro* translation reactions inhibited translation of TS mRNA. This inhibition was specific in that recombinant TS protein had no effect on the *in vitro* translation of mRNA for human chromogranin A, human folate receptor, preplacental lactogen, or total yeast RNA. The inclusion of dUMP, 5-fluoro-dUMP, or 5,10-methylene-tetrahydrofolate in *in vitro* translation reactions completely relieved the inhibition of TS mRNA translation by TS protein. Gel retardation assays confirmed a specific interaction between TS protein and its corresponding mRNA but not with unrelated mRNAs, including human placenta, human β-actin, and yeast tRNA. These studies suggest that translation of TS mRNA is controlled by its own protein end product, TS, in an autoregulatory manner.**

Thymidylate synthase (TS; 5,10-methylenetetrahydrofolate: dUMP *C*-methyltransferase, EC 2.1.1.45) catalyzes the conversion of 2′-deoxyuridine 5′-monophosphate (dUMP) and 5,10-methylenetetrahydrofolate (5,10-methylene-H_4PteGlu, where H_4PteGlu is tetrahydropteroylglutamic acid) to thymidine monophosphate (dTMP) and dihydrofolate (H_2PteGlu) (1). This enzymatic reaction provides the sole intracellular *de novo* source of dTMP, and because of its central role in the synthesis of DNA precursors, TS remains an important target enzyme in cancer chemotherapy (2).

Both the cDNA and corresponding mRNA clones for mouse (3) and human (4) TS have been isolated and sequenced, and these probes have facilitated the analysis of TS structure and expression and the study of the molecular basis of TS regulation. This enzyme has been purified and well characterized from various sources, including bacteria, bacteriophage, yeast, viruses, parasites, and mammals (5–9). TS is a dimeric protein with identical subunits, each ≈35 kDa, and comparison of the predicted primary amino acid sequence of the protein from eight different sources reveals that it is one of the most highly conserved proteins.

Previous studies examining regulation of TS expression have concentrated on cell-cycle-directed events. Various investigators have shown that maximal TS activity occurs during periods of active DNA synthesis (10–12). Moreover, this increase in TS enzyme levels that arises as cells enter S phase appears to be regulated at both the transcriptional and posttranscriptional levels (13–15). Takeishi *et al.* (4) also suggested the possibility of translational regulation of TS expression given the theoretical potential of three interconvertible secondary structures, each containing a stem–loop structure in the 5′ untranslated region (5′ UTR) of the human TS mRNA. At the present time, however, relatively little is known regarding the biological importance of translational regulation of TS mRNA.

Recently, there has been an increased interest in translational regulatory mechanisms (16–20). Biosynthetic regulation of the intracellular iron-storage protein ferritin by iron represents one of the best-characterized examples of translational regulation. Detailed studies have shown that ferritin mRNA contains a specific iron-responsive element within its 5′ UTR to which a 90-kDa cytosolic protein binds (21–26). It is this specific protein–mRNA interaction that appears to control the translational process.

In the present study, we determined whether expression of TS protein was controlled at a translational level by using the cell-free isolated rabbit reticulocyte model system. Our results indicate that translation of TS mRNA is inhibited in the presence of purified recombinant human TS protein. Further experiments using an *in vitro* gel retardation assay provide evidence that TS protein interacts specifically with its own TS mRNA. These findings suggest that translation of TS mRNA is controlled by its own protein product TS in an autoregulatory manner.

MATERIALS AND METHODS

Preparation of Plasmid Constructs and *in Vitro* mRNA Transcripts. The human TS cDNA clone pcHTS-1 was originally isolated and sequenced from a human fibroblast library by Takeishi and colleagues (4) and was a gift from T. Seno (Saitama-ken Research Center, Saitama, Japan). This clone was excised from the phage M13 vector at the *Stu* I and *Xho* I restriction sites and cloned into pGEM-4Z plasmid (Promega). The sequence was confirmed by using the dideoxynucleotide method of Sanger *et al.* (27). Purified pGEM-4Z/pcHTS-1 plasmid was linearized with *Hin*dIII to give pcEHTS-1. Human chromogranin A cDNA was a gift from Lee Helman (Pediatric Branch, National Cancer Institute) (28). The human folate receptor cDNA was isolated and sequenced as described by Elwood (29). All three of these cDNAs were transcribed into their corresponding mRNAs by using SP6 RNA polymerase and the supplied reagents (Promega) according to the Promega protocol. Yeast and human preplacental lactogen RNAs were supplied in the protein-processing translation system (New England Nuclear). All mRNA transcripts were evaluated on a 1.5% agarose/formaldehyde gel to verify their integrity and size. Labeled

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Abbreviations: TS, thymidylate synthase; 5,10-methylene-H_4PteGlu, 5,10-methylenetetrahydrofolate (5,10-methylenetetrahydropteroylglutamic acid; UTR, untranslated region; FdUMP, 5-fluoro-dUMP.

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RNA transcripts were made by inclusion of [α-^{32}P]CTP (specific activity, 200 Ci/mmol).

TS Protein Purification. Homogeneously purified human recombinant TS protein (specific activity, 0.7 unit/mg) was a gift from Daniel Santi (Department of Biochemistry and Biophysics, University of California, San Francisco). This protein was purified by the method of Davisson *et al.* (30). Homogeneously pure TS protein was used in the *in vitro* translation studies, since the absence of RNase degradative activity was a critical factor in demonstrating the specific inhibitory effects of TS protein on TS mRNA translation. In addition, larger quantities of the human TS protein were partially purified by using ammonium sulfate fractionation (30–65%) followed by affinity chromotography with an Affi-Gel Blue Sepharose column as described by Lu *et al.* (31). The specific activity of this partially purified TS was 0.10 unit/mg of protein, which is less pure than the homogeneously purified TS protein by a factor of 7.

***In Vitro* Translation.** Translation reaction mixtures (final volume, 30 μl) containing rabbit reticulocyte lysate (10 μl), 66 μM potassium acetate, 0.5 mM magnesium acetate, 50 μCi (1 μCi = 37 kBq) of [^{35}S]methionine, and translation cocktail (13 μl), all supplied in a protein-processing translation system (New England Nuclear), were incubated with the respective RNA transcripts at 37°C for 60 min, and the products were analyzed by SDS/PAGE (15% acrylamide) according to the method of Laemmli (32). Unincorporated label was removed by soaking the gel in 100 ml of 40% (vol/vol) methanol/10% (vol/vol) glacial acetic acid for 20 min. The solution was changed three times, following which the gel was soaked in 250 ml of Enlightening (New England Nuclear) and 3% glycerol for 2 hr at room temperature. After drying for 2 hr, the translation products were identified by autoradiography. Quantitation of signal intensities was performed by densitometer scanning (Beckman DU-65 spectrophotometer).

Western Immunoblot Analysis. *In vitro* translation reactions were incubated with pcEHTS-1 RNA in the absence or presence of purified human recombinant TS protein at 37°C for 60 min. The products were resolved by SDS/PAGE (15% acrylamide). The gel was then electroblotted onto a nitrocellulose membrane with an LKB 2117 Multiphor II electrophoresis unit set at 200 mA for 2 hr. Antibody staining was performed with a TS polyclonal primary antibody at a 1:1000 dilution in Blotto buffer. A horseradish-conjugated antibody at a dilution of 1:1000 in Blotto buffer was used as the secondary antibody.

RNA–Protein Binding Assay. RNA–protein binding experiments were performed as described by Leibold and Munro (22). Labeled RNAs (0.5–1.0 ng) were incubated with partially purified human recombinant TS (0.8 μg of TS protein) for 30 min at 37°C. RNase T1 (1 unit, Boehringer Mannheim) was then added for 10 min followed by incubation with heparin (5 mg/ml, Fisher Scientific) for an additional 10 min. Unlabeled competitor RNAs were mixed with labeled probes prior to the addition of TS protein. Samples were electrophoresed in a 4% nondenaturing acrylamide gel (acrylamide/methylenebisacrylamide ratio, 60:1), dried, and visualized by autoradiography.

RESULTS

Demonstration of Translational Inhibition *in Vitro*. Translation of the rabbit lysate in the absence of exogenous mRNA transcript did not yield a protein product during exposures typically required to visualize the protein products from exogenously added mRNAs (Fig. 1, lane A). Longer exposures of the autoradiograph in the absence of exogenous mRNA did eventually reveal a protein product resolving at approximately 47 kDa. When 0.25 μg of TS mRNA transcript was included in the rabbit reticulocyte reaction mixture, a band representing TS protein at 35 kDa was observed (Fig. 1, lane B). A less intense band was also observed at ≈27 kDa that appears to represent a TS protein degradation product. Translation of TS mRNA was almost completely inhibited by the addition of 0.4 μg of human recombinant TS (Fig. 1, lane C). The addition of 1 μg of bovine serum albumin did not inhibit the translation of TS mRNA, suggesting that the inhibitory effect of TS protein was protein-specific. The specificity of TS protein inhibition was further supported by experiments in which the addition of exogenous TS protein to reaction mixtures containing unrelated mRNA transcripts, such as human chromogranin A, yeast, human folate receptor, and human preplacental lactogen, did not alter their respective translational efficiencies (Fig. 1, lanes E–L).

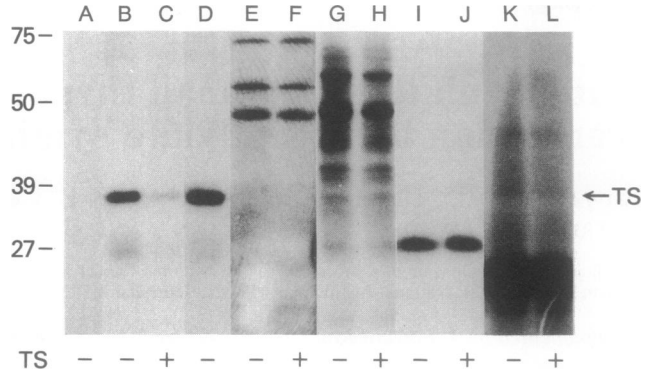

FIG. 1. Specificity of inhibition by TS enzyme of human TS mRNA translation *in vitro*. Translation reactions containing rabbit reticulocyte lysate (10 μl) were incubated with no exogenous mRNA (lane A), 0.25 μg of TS mRNA (lanes B–D), 0.25 μg of human chromogranin A mRNA (lanes E and F), 0.25 μg of yeast mRNA (lanes G and H), 0.25 μg of human folate receptor mRNA (lanes I and J), and 0.25 μg of human preplacental lactogen mRNA (lanes K and L). Purified human recombinant TS protein (0.4 μg) was included in the reaction mixtures where indicated. In lane D, bovine serum albumin (1 μg) was added to the reaction mixture. Translation reactions were incubated at 37°C for 60 min, and protein products were analyzed by SDS/PAGE (15% acrylamide). Sizes are shown in kDa.

We next determined the effect of varying the concentration of TS mRNA on translational efficiency in the absence and presence of exogenous TS protein. Saturation of translation was found to occur at TS mRNA concentrations > 0.4 μg (data not shown). TS synthesis was linearly dependent upon the concentration of TS mRNA up to 0.2 μg (Fig. 2 *Left*, lanes A–C). The addition of exogenous TS protein (0.4 μg) to reaction mixtures containing 0.2, 0.1, and 0.05 μg of TS mRNA resulted in near complete inhibition of TS protein synthesis (Fig. 2 *Left*, lanes D–F, respectively). We also determined the dose-dependent effect of TS protein inhibition. When varying concentrations of TS protein (0.4, 0.2, and 0.1 μg) (Fig. 2 *Right*, lanes B–D, respectively) were incubated with 0.4 μg of TS mRNA, there was a respective 45%, 24%, and 12% inhibition of translation as compared with control TS mRNA translation (Fig. 2 *Right*, lane A).

The effects of occupancy of either the nucleotide or folate binding sites of TS enzyme on its subsequent interaction with TS mRNA were also examined. The normal substrates for TS are the nucleotide dUMP and the reduced folate 5,10-methylene-H_4PteGlu. 5-Fluorouracil, an important antineoplastic agent, is metabolized intracellularly to the nucleotide 5-fluoro-dUMP (FdUMP), which binds to TS to form a binary complex (33). In the presence of the reduced substrate, 5,10-methylene-H_4PteGlu, a tight ternary complex is formed. When TS mRNA (0.2 μg) was incubated in the absence (Fig. 3, lane A) or presence (Fig. 3, lane B) of exogenous TS protein (0.4 μg), significant inhibition of translation in the

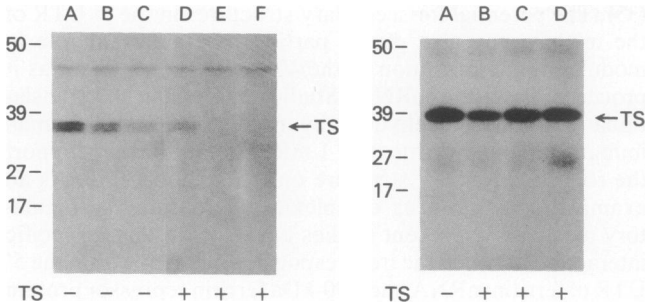

FIG. 2. Dose-dependent effect of inhibition by TS enzyme of human TS mRNA translation *in vitro*. (*Left*) Various concentrations of human pcEHTS-1 mRNA transcript, 0.2 (lanes A and D), 0.1 (lanes B and E), and 0.05 μg (lanes C and F), were included in the *in vitro* translation reaction mixtures. Purified human recombinant TS (0.4 μg) was added to the reaction mixtures where indicated. Translation reactions and product analyses were performed as described. (*Right*) pcEHTS-1 mRNA transcript (0.5 μg) was included in the absence (lane A) and presence (lanes B–D) of decreasing concentrations of human recombinant TS: 0.4 μg (lane B), 0.2 μg (lane C), and 0.1 μg (lane D). Translation reaction mixtures were processed as outlined. Quantitation of signal intensities was performed by densitometer scanning (Beckman DU-65 spectrophotometer) and expressed as percent of control (lane A). Sizes are shown in kDa.

reaction containing exogenous TS protein was again noted. Addition of the nucleotides FdUMP, dUMP, or the reduced folate 5,10-methylene-H_4PteGlu to reaction mixtures containing both TS protein and TS mRNA completely prevented the inhibition of translation by exogenous TS (Fig. 3, lanes C–E). When 5,10-methylene-H_4PteGlu was combined with either dUMP or FdUMP in reaction mixtures containing TS protein and TS mRNA, there was no further increase in TS synthesis when compared with the effect of the individual substrates alone. In contrast, addition of these substrates to reactions containing only TS mRNA did not alter TS synthesis (data not shown).

Since these findings showed that TS mRNA translation was controlled by the presence of exogenous human recombinant TS protein, we next determined whether the TS protein synthesized in the *in vitro* rabbit reticulocyte lysate system had a similar inhibitory effect on translation. When an aliquot of newly translated TS protein was subsequently added to a fresh reaction mixture containing only TS mRNA, there was no change in TS mRNA translation compared with the addition of rabbit reticulocyte lysate alone (data not shown). Although the denatured form of this *in vitro*-made TS protein resolves at a molecular weight of 35 kDa, as does the human recombinant TS protein, it is conceivable that post-translational modification or processing of this TS protein product is altered in the rabbit reticulocyte lysate. Additional investigation using both a radioligand and spectral assay system for quantitating TS enzyme activity (30, 34) revealed that the *in vitro*-made TS protein, unlike the human recombinant TS, was catalytically inactive. Thus, alterations in the *in vitro*-translated TS protein may lead to an inability to interact with the TS mRNA, as was suggested by the inability of this protein to inhibit the *in vitro* translation of TS mRNA.

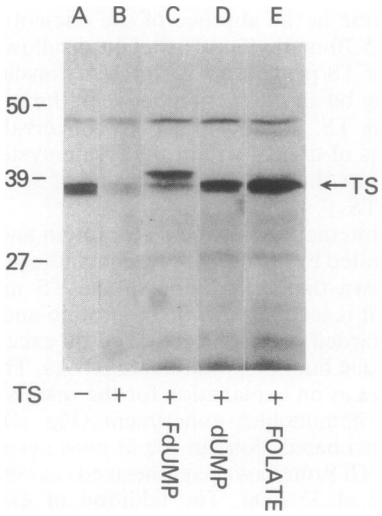

FIG. 3. Reversal of TS mRNA translational inhibition by FdUMP, dUMP, and 5,10-methylene-H_4PteGlu. pcEHTS-1 mRNA transcript (0.2 μg) alone (lane A) or accompanied by purified human recombinant TS (0.4 μg) where indicated (lanes B–E), along with 30 μM FdUMP (lane C), 30 μM dUMP (lane D), or 300 μM 5,10-methylene-H_4PteGlu (lane E) were included in reaction mixtures that were incubated at 37°C for 60 min. Translation products were analyzed by SDS/PAGE (15% acrylamide), and autoradiography was as described. Sizes are shown in kDa.

Western Immunoblot Analysis. To further verify that the protein product resolving at 35 kDa represented the monomeric form of TS, we analyzed the translation products by Western immunoblot using a rabbit-derived TS polyclonal antibody. Equal amounts of rabbit lysate containing no exogenous mRNA (Fig. 4, lane A) or 0.1 μg of TS mRNA in the absence (Fig. 4, lane B) and presence (Fig. 4, lane C) of 0.2 μg of exogenous TS protein were incubated for 1 hr and then resolved on a 15% SDS/polyacrylamide gel electrophoresis. As seen in the reaction mixture containing no mRNA transcript (Fig. 4, lane A), several bands were stained by the TS polyclonal antibody. Since we used a polyclonal antibody that was derived from rabbits, these bands reflect cross-reactivity of the polyclonal antibody with the endogenous proteins present in the rabbit reticulocyte lysate. There was intense staining at 35 kDa representing TS protein in the reaction mixtures containing only TS mRNA (Fig. 4, lane B). In contrast, with the addition of exogenous TS, TS protein synthesis was significantly inhibited as demonstrated by a marked decrease in staining at 35 kDa (Fig. 4, lane C). Examination of the reaction mixture containing TS mRNA and TS protein (Fig. 4, lane C) revealed a band with less than the expected intensity given the exogenously added TS protein. The TS polyclonal antibody recognized human recombinant TS protein when it was included, by itself, in the *in vitro* translation mixture (Fig. 4, lane E). In contrast to what was observed with the reaction containing TS mRNA and TS protein, when human folate receptor mRNA and exogenous TS protein were included in the *in vitro* translation mixture, the presence of exogenous TS protein is observed (Fig. 4, lane D). Taken together, these findings suggest that there exists a specific interaction between TS protein and its corresponding mRNA resulting in an SDS-stable complex that is unable to enter into the 15% SDS–PAGE gel. This is

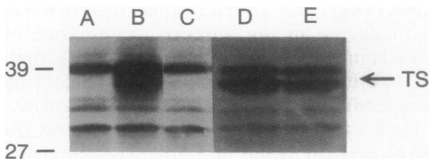

FIG. 4. Western immunoblot analysis of inhibition by TS enzyme of TS mRNA translation *in vitro*. Translation reactions included no exogenous mRNA (lane A), 0.1 μg of pcEHTS-1 mRNA in the absence (lane B) or presence of 0.2 μg of TS protein (lane C), 0.1 μg of human folate receptor mRNA in the presence of 0.2 μg of TS protein (lane D), and 0.2 μg of TS protein alone (lane E). Translation reaction mixtures were incubated at 37°C for 60 min, and the protein products were resolved by SDS/15% PAGE. The gel was electroblotted onto a nitrocellulose membrane, and antibody staining was performed as described.

in contrast to the *in vitro*-made TS protein, which lacks catalytic activity and appears unable to interact with TS mRNA, allowing for the presence of the TS protein product at 35 kDa.

Demonstration of Specific RNA–Protein Binding. The ability of TS protein to specifically interact with TS mRNA was determined by means of a gel retardation assay. When an RNA probe containing the full-length TS mRNA transcript was incubated with partially purified TS protein, a complex was formed that resulted in the retarded migration of the labeled RNA in the gel (Fig. 5*A*, second TS lane; Fig. 5*B*, lane 2). In contrast, a probe derived from the first 413 bases of human β-actin mRNA did not form such a complex (Fig. 5*A*, second actin lane). When the protein extract was pretreated with proteinase K (1 μg/ml) at 37°C for 30 min, the complex was not present, confirming that binding required the presence of intact protein (Fig. 5*B*, lane 8). No complex was formed when the TS mRNA probe was incubated with bovine serum albumin (Fig. 5*B*, lane 9), suggesting that the interaction between TS mRNA and TS protein is specific.

As further support of the specificity of the retardation complex, competition studies were performed. Addition of unlabeled TS mRNA in excess by 5-fold (Fig. 5*B*, lane 3), 50-fold (Fig. 5*B*, lane 4), and 500-fold (Fig. 5*B*, lane 5) inhibited the formation of complex in a dose-dependent manner. In contrast, high concentrations (500 ng) of the nonspecific competitors human placenta RNA (Fig. 5*B*, lane 6) and yeast tRNA (Fig. 5*B*, lane 7) were not competitive.

DISCUSSION

Translation of RNA is a complex process that may be controlled at several key steps. Initiation of translation is considered to be a critical event in this process and requires the interaction between ribosomal subunits, translation initiation factors, the cap of mRNA, and the 5′ UTR of mRNA

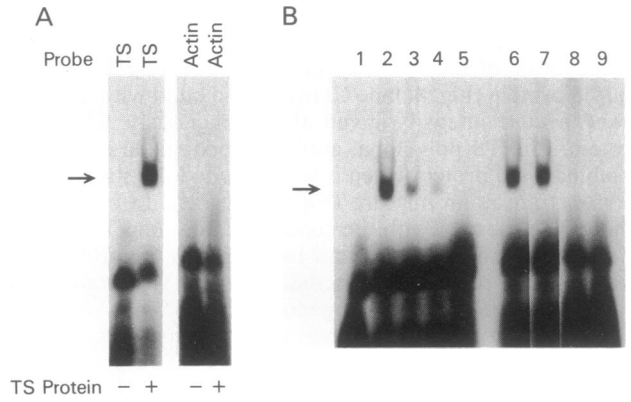

FIG. 5. Specific binding of TS protein to TS mRNA. (*A*) Labeled human TS RNA (1 ng; lanes TS) and human β-actin RNA (1 ng; lanes Actin) were incubated in the absence or presence of 800 ng of partially purified human recombinant TS protein (second lane of each pair). Samples were electrophoresed in a nondenaturing 4% acrylamide gel. The specific complex is indicated by the arrow. (*B*) Labeled TS RNA (1 ng) was incubated in the absence (lane 1) or presence (lane 2) of 800 ng of partially purified human recombinant TS protein. TS protein (800 ng) was also included in reaction mixtures represented in lanes 3–7. Competition studies were performed with 5-fold (lane 3), 50-fold (lane 4), and 500-fold (lane 5) excess unlabeled TS RNA and 500-fold excess of either human placenta RNA (lane 6) or yeast tRNA (lane 7). TS protein (800 ng) was preincubated with proteinase K (1 μg/μl) for 30 min and then included in a reaction mixture containing labeled TS RNA (lane 8). Labeled TS RNA (1 ng) was also incubated with bovine serum albumin (1 μg) (lane 9). Samples were electrophoresed in a nondenaturing 4% acrylamide gel and visualized by autoradiography. The specific complex is indicated by the arrow.

(35). The potential for secondary structures in the 5′ UTR of the mRNA may also play a particularly important role in modulating the migration of the 40S ribosomal subunit as it proceeds along the mRNA. Studies examining the translational control of aldehyde dehydrogenase (17) and human immunodeficiency virus type 1 mRNAs (36) further support the role of secondary structure on translational rate. As an example of the growing complexity of translational regulatory mechanisms, recent studies have shown that a specific interaction between the iron-responsive element within the 5′ UTR of ferritin mRNA and a 90-kDa ferritin repressor protein exists that is directly responsible for the translational control of ferritin (37).

The results of our study show that TS mRNA translation is inhibited in the presence of TS protein and that there is a specific interaction of TS protein with its mRNA. This inhibition is specific in that the translation of other unrelated mRNA transcripts remains unaffected by TS protein. Moreover, our findings suggest that a proportional relationship exists between the concentration of TS mRNA and the concentration of TS protein required to inhibit translation. Inclusion of any one of the substrates for TS, including FdUMP, dUMP or 5,10-methylene-H_4PteGlu, along with exogenous TS protein completely restores translation of TS mRNA, a finding that further supports the specificity of this inhibition and suggests that, for the TS protein to interact with its mRNA, a certain conformational state may be required. The inability of the *in vitro*-made TS protein, a protein devoid of catalytic activity, to interact with TS mRNA provides additional evidence that a biologically active state of the protein is important.

Previous studies have described the catalytic reaction at TS as an ordered process where the nucleotide dUMP binds first followed by binding of the folate cofactor (38). Galivan *et al.* (39) were unable to show binding of the folate cofactor to the bacterial enzyme in the absence of the nucleotide substrate. While similar studies have not been performed with the human enzyme, other mechanisms have been proposed in which reverse or random ordering may occur (31, 40). Additional data from molecular modeling studies suggest that the folate substrate may interact, albeit noncovalently, with TS enzyme in the absence of the nucleotide (41). Our findings that 5,10-methylene-H_4PteGlu disallows the inhibitory effects of TS protein on TS mRNA translation suggest that there may be an interaction between the reduced folate substrate and TS. Moreover, it is conceivable that the concentrations of dUMP within the lysate system are sufficient to facilitate the subsequent binding of 5,10-methylene-H_4PteGlu to TS.

A specific interaction between TS protein and TS mRNA was demonstrated by our RNA–protein binding experiments. We have shown that retardation of the TS mRNA probe occurs when it is incubated with TS protein and that formation of the retarded complex is blocked by excess unlabeled TS mRNA probe but not by unrelated RNAs. These findings may also serve as an explanation for the results observed in our Western immunoblot experiment (Fig. 4). When TS mRNA was incubated alone in the *in vitro* system, a significant level of TS protein was synthesized, as revealed by an intense signal at 35 kDa. The addition of exogenous TS protein to this same reaction mixture resulted in almost complete disappearance of the signal at 35 kDa. Although this finding is consistent with inhibition of TS mRNA translation by exogenous TS protein, there is an apparent absence of the exogenous TS protein itself on this Western blot. However, given the specific interaction between TS mRNA and TS protein resulting in a retarded complex, it is probable that under the conditions of the gel used in this Western analysis (15% polyacrylamide) as opposed to the 4% nondenaturing

gel used in the RNA–protein binding experiments, any complex that formed would be unable to enter the gel.

Thus, our results suggest that translation of TS mRNA is autoregulated by its own protein end product, TS. Furthermore, this translational regulation correlates with binding of the TS protein to the TS mRNA. It would appear, then, that the intracellular level of TS protein is a critical determinant of TS mRNA translation. This model may be used to explain the findings observed in various *in vitro*, *in vivo*, and clinical studies that describe increases in TS levels in neoplastic cells following acute exposures to 5-fluorouracil (34, 42–44). Fluoropyrimidine treatment results in the intracellular accumulation of the pyrimidine nucleotides FdUMP and dUMP. Each of these nucleotides can interact with TS protein to decrease the intracellular level of unbound TS, which then results in enhanced TS mRNA translation. Thus, these findings appear to have biological relevance in that they suggest (*i*) a means whereby the level of this protein is finely controlled within a given cell and (*ii*) a mechanism for malignant cells to protect themselves in response to a cytotoxic stress such as 5-fluorouracil.

This form of autoregulatory translational control has not been previously described in eukaryotes to our knowledge. However, Bernardi and Spahr (45) have reported that the coat protein of bacteriophage R17 represses *in vitro* translation of the R17 replicase gene. Further studies have now identified the specific sequence and size of the RNA binding site as well as the importance of secondary structure in this protein–mRNA interaction (46–48). Additional work is required to more completely characterize the specific interaction between TS mRNA and its protein product TS. It will also be important to determine whether translational regulation of human TS mRNA occurs in the *in vivo* setting. However, these initial studies provide new insights into the molecular mechanisms underlying the regulation of TS expression and contribute to the growing knowledge regarding regulation of translation.

The authors thank Drs. Richard Klausner and Joe Harford for their insightful and helpful discussions, Dr. Daniel Santi for his generous gift of the purified human recombinant TS protein, and Kathy Moore for her editorial assistance in the preparation of this manuscript.

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