Regulation of Folate Reductase Synthesis in Sensitive and Methotrexate-resistant Sarcoma 180 Cells

IN VITRO TRANSLATION AND CHARACTERIZATION OF FOLATE REDUCTASE mRNA*

(Received for publication, May 24, 1976)

RODNEY E. KELLEMS,‡ FREDERICK W. ALT,§ AND ROBERT T. SCHIMKE
From the Department of Biological Sciences, Stanford University, Stanford, California 94305

A highly specific assay for folate reductase mRNA activity from Sarcoma 180 cells was developed using the rabbit reticulocyte lysate protein synthesizing system. Quantitation of in vitro folate reductase synthesis was accomplished by direct immunoprecipitation from lysate reactions. The in vitro labeled folate reductase was synthesized in a linear response to a wide range of RNA concentrations, migrated as a single prominent radioactive species upon polyacrylamide gel electrophoresis, and was indistinguishable from authentic 14C-labeled folate reductase on the basis of molecular weight and immunotitration with anti-folate reductase γ-globulin.

The assay was used to quantitate folate reductase mRNA activity in various cell lines and under several conditions known to affect folate reductase synthesis. These included (a) sensitive and methotrexate-resistant Sarcoma 180 cells, (b) two lines of resistant cells having different relative rates of folate reductase synthesis, (c) growth of methotrexate-resistant cells in the absence of methotrexate, and (d) growth phase. The results indicate that the relative rate of folate reductase synthesis in each case can be explained solely by the level of translatable folate reductase mRNA.

The use of poly(U)-Sepharose and sucrose gradient fractionation procedures indicated that folate reductase mRNA contains poly(A) and has a sedimentation coefficient of approximately 14 S. These two fractionation steps were combined to achieve an approximately 90-fold purification of folate reductase mRNA over total cytoplasmic RNA.

Recently we began a detailed analysis of the molecular mechanisms involved with the overproduction of folate reductase by certain lines of highly methotrexate-resistant Sarcoma 180 cells (1, 2). The use of such mammalian cell variants which possess well defined regulatory alterations presents a promising approach to defining the molecular events underlying the regulation of gene expression in eukaryotic cells. Folate reductase constitutes as much as 6 to 8% of the soluble protein in these cells representing an increase of more than 200-fold over the levels of enzymatically active and immunologically cross-reactive folate reductase present in sensitive cells (1, 2). The purified enzymes from sensitive and resistant cells were indistinguishable with regard to numerous physical, kinetic, and immunochemical properties (1-4) suggesting that folate reductase was not altered in resistant cells. The use of highly specific immunological and protein purification procedures enabled us to clearly show that the increased levels of folate reductase in several lines of methotrexate-resistant Sarcoma 180 cells are entirely the result of corresponding increases in the relative rate of folate reductase synthesis (1). Furthermore, other factors shown to affect folate reductase levels did so by regulating the relative rate of folate reductase synthesis (1).

In this report we describe an assay for folate reductase mRNA activity using the rabbit reticulocyte lysate protein synthesizing system. The assay was used to quantitate folate reductase mRNA activity in cells under a variety of conditions affecting the rate of folate reductase synthesis. In this way the role of folate reductase mRNA levels in the regulation of these events was clearly defined. Also presented are an initial characterization and partial purification of folate reductase mRNA using poly(U)-Sepharose and sucrose gradient fractionation procedures.

EXPERIMENTAL PROCEDURES

Materials

The procedures used for the preparation of 14C-labeled folate reductase, anti-folate reductase γ-globulin, as well as the sources of most of the reagents have been given (1). Poly(U)-Sepharose was purchased from Pharmacia, and heparin was obtained from Riker Laboratories.

Cell Culture

The Sarcoma 180 cell line and methotrexate-resistant sublines (AT-3000 and R-1) were cultured as described previously (1). Various properties of these cell lines have been reported (1-4).
Denaturing Conditions, 

The relative rate of folate reductase synthesis was determined as described previously (1) by direct immunoprecipitation of folate reductase from extracts of pulse-labeled cells. Incorporation of radioactivity into folate reductase is expressed as a percentage of total trichloroacetic acid-precipitable radioactivity in cell extracts.

RNA Preparation

RNA was usually extracted from several grams of early to mid-log phase cells, except when noted otherwise. Cells were grown in roller bottles and received fresh media 3 to 5 h before harvesting. Immediately prior to harvest, cells were rinsed twice with ice-cold Hank's balanced salts solution, then scraped from roller bottles with a rubber policeman, and washed three additional times with Hank's balanced salts solution. All subsequent operations were carried out at 0-4°C. Cell pellets were homogenized in 7 volumes of 25 mM Tris/Cl, pH 7.1, 25 mM NaCl, 10 mM MgCl₂, 5% (w/v) sucrose, and 1 mg/ml of heparin by five strokes with a Dounce homogenizer. One cell volume of 10% (v/v) Triton X-100, 10% (w/v) deoxycholate was added, and the homogenate was given five additional strokes. The homogenate was centrifuged for 10 min at 27,000 x g max, and to the supernatant was added 0.2 volume of 10% (w/v) sodium dodecyl sulfate, 50 μM EDTA, and 100 mM Tris/Cl, pH 7.1. RNA was obtained from this fraction by four or five successive phenol/chloroform extractions (5) is designated as cytoplasmic RNA and was prepared for assay in the rabbit reticulocyte lysate system essentially as described by McKnight and Schimke (6). All glassware and solutions used in the preparation and fractionation of RNA were autoclaved for 25 min.

Generally, 2 mg of RNA were obtained from 1 g of packed cells. In our calculations we assumed that 1 A₂₆₀ unit of RNA was equivalent to 50 μg (6).

Assay for Folate Reductase mRNA Activity

The unfractionated rabbit reticulocyte lysate protein synthesizing system (7, 8) was used to quantitate folate reductase mRNA activity. The conditions used in our assay were the same as those described by Schimke et al. (9) for the determination of ovalbumin mRNA activity, except that nonradioactive carrier leucine was omitted from the reaction mixture. Following incubation for 1 h at 37°C, the 250-μl lysate reaction was terminated by addition of 40 μl of 10% (v/v) Triton X-100, 10% (w/v) sodium deoxycholate, and 110 μl of 0.1 M leucine and was centrifuged for 10 min at 12,000 x g max. Labeled folate reductase was immunoprecipitated from 100-μl aliquots of the supernatant as described previously (1), except that only 0.1 to 0.25 μg of 14C-labeled folate reductase was used as carrier and the volume of the immunoprecipitation reaction was adjusted to 250 μl by addition of 0.1 M leucine. Incorporation into folate reductase was determined as the difference in radioactivity appearing in immunoprecipitates from lysate reactions that had received RNA and control lysate reactions to which no RNA had been added. Immunoprecipitates from 100 μl of control lysates usually gave 200 to 300 cpm. Final values are expressed as total incorporation of [3H]leucine into immunoprecipitable folate reductase.

Sedimentation Analysis of Folate Reductase mRNA Activity

Cytoplasmic RNA from AT-3000 cells was examined by sedimentation through sucrose gradients prepared under denaturing or non-denaturing conditions.

Nondenaturing Conditions—RNA samples were prepared in 1% (w/v) sodium dodecyl sulfate, 5 mM EDTA, and 1 mM Tris/Cl, pH 7.1, and, when specified, heated for 10 min at 88°C and rapidly cooled in an iced ice bath. This procedure has been effectively used to disaggregate RNA (5, 10-12). Samples were layered on isokinetic sucrose density gradients prepared in 1% (w/v) sodium dodecyl sulfate, 5 mM EDTA, and 10 mM Tris/Cl, pH 7.1, and centrifuged in an SW 41 rotor (Beckman) for 6.5 h at 41,000 rpm. Gradients were prepared as outlined by McCarty et al. (13) for the SW 41 rotor and run at 20°C. The initial concentration of sucrose at the top of the gradient (i.e., C₄ₓₜₖ (13)) was 5% (w/v).

Denaturing Conditions—Samples were prepared in 85% formamide, 1.0 mM Tris/Cl, pH 8.0, and 1 mM EDTA and heated for 2 min at 88°C. The RNA was applied to a linear sucrose gradient and centrifuged for 30 h in an SW 41 rotor using the conditions given by Macnaughton et al. (13).

After centrifugation the gradients were separated into 0.6-ml fractions with an Isco model 640 density gradient fractionator equipped with an ultraviolet flow monitor. In most cases, the optical density of each fraction was determined independently. Each fraction was adjusted to contain 0.3 M NaCl and from 20 to 40 μg of hen oviduct carrier mRNA (i.e., poly(A) minus RNA containing no detectable folate reductase mRNA activity). RNA was precipitated overnight at -20°C after addition of 2.0 volumes of ethanol. The precipitates were collected by centrifugation and washed twice with 70% ethanol 0.1 M NaCl. The washed precipitates were dried by lyophilization, resuspended in a minimum volume of H₂O, and equal aliquots from each fraction were assayed for folate reductase mRNA activity. In most cases nearly all of the folate reductase mRNA activity applied to the gradient was recovered in the gradient fractions.

Get Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed as described previously (1).

RESULTS

Quantitation of Folate Reductase mRNA Activity

Folate Reductase mRNA Levels in Sensitive and Methotrexate-resistant Sarcoma 180 Cells—The overproduction of folate reductase by the methotrexate-resistant AT-3000 line of Sarcoma 180 cells is due to a specific increase in the relative rate of folate reductase synthesis (1). In order to determine if this results from an increased level of folate reductase mRNA activity, we assayed cytoplasmic RNA from sensitive and methotrexate-resistant cells for the ability to direct the synthesis of folate reductase in the rabbit reticulocyte lysate protein synthesizing system. Folate reductase synthesis was quantitated by direct immunoprecipitation of the in vitro labeled enzyme. A linear response in folate reductase synthesis was obtained with up to 75 μg of added RNA, thus enabling the assay to be used for quantitative purposes over a wide range of RNA concentrations. With this assay, it was possible to show (Fig. 1) that cytoplasmic RNA from methotrexate-resistant cells had a considerably higher level of folate reductase mRNA activity than cytoplasmic RNA from sensitive cells. As a control experiment we determined that RNA from a mixed population of resistant and sensitive cells (containing an equal quantity of each) had an intermediate level of folate reductase mRNA activity (Fig. 1). This result indicates that the difference in folate reductase mRNA activity between resistant and sensitive cells is not due to either an inhibitor of in vitro folate reductase synthesis or a high ribonuclease activity occurring in the sensitive cell extracts. We conclude from these results that the increased rate of folate reductase synthesis by resistant...
Regulation of Folate Reductase Synthesis

6989

cells is due, at least in part, to elevated levels of translatable folate reductase mRNA. The quantitative aspect of this conclusion is considered in more detail below.

The apparent low level of stimulation of incorporation into folate reductase by RNA from sensitive cells (~8 cpm/µg of RNA) is quantitatively similar to that resulting from the addition of hen oviduct rRNA, i.e. poly(A) minus RNA, to lysate reactions (data not shown). This level of incorporation probably represents nonspecific radioactivity associated with the addition of a heterologous RNA to the lysate reaction. Therefore, a more sensitive comparison of the folate reductase mRNA activity of RNA from sensitive and methotrexate-resistant cells was accomplished by electrophoretic analysis of immunoprecipitates from lysate reactions that received comparable amounts of RNA from either cell type. The radioactivity immunoprecipitated from lysate reactions prepared with RNA from resistant cells electrophoresed as one prominent band, in contrast to the multiple bands immunoprecipitated from lysate reactions prepared with RNA from sensitive cells (Fig. 2b). Thus, folate reductase mRNA activity was not detectable in preparations of RNA from sensitive cells. The electrophoretic analysis of folate reductase immunoprecipitates (Fig. 2) was sufficiently sensitive to have detected a difference of approximately 100-fold between the level of folate reductase mRNA activity in cytoplasmic RNA from resistant and sensitive cells.

Immunotitration of in Vitro Labeled Folate Reductase and Authentic 14C-labeled Folate Reductase—The folate reductase synthesized in vitro was compared immunologically with authentic 14C-labeled folate reductase by determining the amount of each that was immunoprecipitated with various concentrations of anti-folate reductase γ-globulin. A constant ratio of 3H:14C was obtained for each immunoprecipitate and, as shown in Fig. 3, the same relative percentage of the in vitro 3H-labeled and authentic 14C-labeled folate reductase was immunoprecipitated at each γ-globulin concentration. These results demonstrate that the anti-folate reductase γ-globulin was unable to distinguish between the in vivo and in vitro labeled enzymes, thus indicating their antigenic similarity.

Levels of Folate Reductase mRNA Activity in Cells with Different Rates of Folate Reductase Synthesis—An interesting feature of methotrexate-resistant Sarcoma 180 cells is that growth for extended periods of time in the absence of methotrexate results in a continual decline in the level of enzymatically active and immunologically cross-reactive folate reductase that is correlated with a corresponding decrease in the rate of folate reductase synthesis (1). This property of resistant cells was characterized in more detail by comparing the rates of folate reductase synthesis with the levels of folate reductase mRNA activity in two lines of resistant cells (AT-3000 and a clonally derived subline, R-1) as a function of growth in the presence or absence of methotrexate (Table I). R-1 cells grown in methotrexate have lower rates of folate reductase synthesis and lower levels of folate reductase mRNA activity than those observed for AT-3000 cells. However, growth of either line of resistant cells in the absence of methotrexate resulted in a continual drop in both folate reductase synthesis and mRNA activity to levels eventually approaching those of sensitive cells (Table I).

Another factor that influences the regulation of folate reductase synthesis in methotrexate-resistant cells, and sensitive cells as well, is growth phase (1). Folate reductase synthesis...
in AT-3000 cells is at least 6-fold greater in the early logarithmic phase of cell growth than in stationary phase. In order to define the role of folate reductase mRNA activity in this process, we prepared cytoplasmic RNA from AT-3000 cells that were harvested from logarithmic and stationary phase cultures and determined the levels of folate reductase mRNA activity. A typical experiment is presented in Table II which shows that folate reductase synthesis in stationary phase cells had decreased more than 6-fold compared to that of cells in logarithmic growth. Stationary phase cells had similar decline in the level of folate reductase mRNA activity (Table II). In other growth-phase experiments cultures exhibited intermediate levels of folate reductase synthesis and correspondingly intermediate folate reductase mRNA activities (see Fig. 7).

**Characterization and Partial Purification of Folate Reductase mRNA**

*Sedimentation Analysis of Folate Reductase mRNA Activity—Cytoplasmic RNA from AT-3000 cells was fractionated by sedimentation through sucrose gradients that were prepared either in nondenaturing conditions with sodium dodecyl sulfate (Fig. 4a) or denaturing conditions with 85% formamide (Fig. 4b). This latter set of conditions is expected to define the role of folate reductase mRNA activity in this process, we prepared cytoplasmic RNA from AT-3000 cells that

| Cell line | Generations minus methotrexate | Folate reductase synthesis | Folate reductase mRNA activity |
|-----------|--------------------------------|---------------------------|--------------------------------|
| AT-3000   | 0                              | 5.9                       | 72                             |
|           | 12-15                          | <0.5                      | ND                             |
|           | 147                            | <0.5                      | ND                             |
| R-1       | 0                              | 3.1                       | 55                             |
|           | 12-15                          | 1.2                       | 19                             |
|           | 123                            | <0.5                      | 6                              |

* ND, nondetectable.

**Effect of growth phase on folate reductase mRNA activity**

Methotrexate-resistant AT-3000 cells were harvested from either logarithmic or stationary phase cultures. Stationary phase cultures were prepared by allowing cells to reach confluency and then periodically replacing the old media with fresh media for the following 5 to 7 days. For details concerning the measurement of folate reductase synthesis and mRNA activity see the legend to Table I.

| Growth phase | Folate reductase synthesis | Folate reductase mRNA activity |
|--------------|----------------------------|--------------------------------|
|              | % total                    | cpm/μg                         |
| Logarithmic  | 5.2                        | 85                             |
| Stationary   | 0.8                        | 4                              |

**TABLE II**

**TABLE III**

**Effect of methotrexate on folate reductase mRNA activity**

Methotrexate-resistant AT-3000 cells were harvested from either logarithmic or stationary phase cultures. Stationary phase cultures were prepared by allowing cells to reach confluency and then periodically replacing the old media with fresh media for the following 5 to 7 days. For details concerning the measurement of folate reductase synthesis and mRNA activity see the legend to Table I.

**Fig. 4.** Sedimentation analysis of folate reductase mRNA activity. Cytoplasmic RNA from AT-3000 cells was sedimented through sucrose gradients prepared in either nondenaturing conditions with sodium dodecyl sulfate (a, 500 μg of RNA) or denaturing conditions with 85% formamide (b, 200 μg of RNA). The gradients were fractionated and the optical density and folate reductase mRNA activity of each fraction determined as described under “Experimental Procedures.” The data represent the average of duplicate immunoprecipitations from each lysate reaction. Sedimentation coefficients shown in a and b were positioned on the basis of the equation given by McCarty et al. (13). In b, the positions of HeLa 18 S and 28 S RNA species are shown for reference purposes only and do not necessarily reflect actual sedimentation coefficients under these conditions. Folate reductase mRNA activity, O—O; optical density, ▲—▲; 3H-labeled RNA from HeLa cells, Δ—Δ.
Cytoplasmic RNA from AT-3000 cells was fractionated by poly(U)-Sepharose chromatography. Cytoplasmic RNA from AT-3000 cells (usually 4 to 5 mg) was fractionated by the poly(U)-Sepharose chromatographic procedure employed by Shapiro and Schimke (25), except that (a) the original RNA was heated for 10 min at 68°C without NaCl and rapidly cooled in an ethanol-ice bath before application to the column, and (b) the bound RNA was eluted with 98.6% formamide, 10 mM 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid, pH 7.0, and 1 mM EDTA. The folate reductase mRNA activity of the original (A—A), bound and eluted (○—○), and unbound (■—■) fractions was assayed as described under "Experimental Procedures." Each point represents the average of two independent experiments and are corrected for a nonspecific background of 8 cpm/μg.

### Table III

| Fraction       | RNA (μg) | Folate reductase mRNA activity (cpm/μg) |
|----------------|---------|--------------------------------------|
| Original       | 4.6     | 276 (5.1 x 10^3)                  |
| Unbound        | 4.0     | 59.2 (14.8)                         |
| Bound and eluted| 0.06   | 106 (1760)                         |

**Purification**

- **Total**
- **Specific**
- **Purification**

We have recently examined several factors affecting the regulation of folate reductase synthesis in Sarcoma 180 cells and found that in each case the level of folate reductase was determined solely by the rate of folate reductase synthesis (1). In this report we have sought to define the role of folate reductase mRNA activity levels in these regulatory events. In order to carry out these studies, a convenient and highly specific assay for folate reductase mRNA was developed employing the rabbit reticulocyte lysate protein synthesizing system (7, 8). On the basis of the following considerations we judged the assay to quantitate specifically the in vitro synthesis of folate reductase.

1. The **in vitro** labeled product, synthesized in a linear response to a wide range of RNA concentrations, was immunoprecipitated from lysate reactions by a highly specific antibody to folate reductase γ-globulin and migrated as a single radioactive species upon polyacrylamide gel electrophoresis.

2. The molecular weight of the **in vitro** product was indistinguishable from that of authentic 14C-labeled folate reductase as judged by sodium dodecyl sulfate polyacrylamide gel electrophoresis.

3. Immunotitration of the **in vitro** product in the presence of 14C-labeled folate reductase revealed no antigenic differences detectable by anti-folate reductase γ-globulin.
4. The extent of [3H]leucine incorporation into the in vitro product as a function of added RNA was proportional to the level of folate reductase and the rate of folate reductase synthesis in the cells from which the RNA was derived (see Fig. 7 and below).

Furthermore, in experiments not shown here, we were able to purify in vitro labeled folate reductase from lysate reactions by the use of methotrexate-Sepharose affinity chromatography. However, the usefulness of this procedure as a routine method for measuring folate reductase synthesis was hampered by poor recoveries.

Folate reductase constitutes as much as 6 to 8% of the soluble protein in mid-logarithmic phase methotrexate-resistant AT-3000 cells. This striking overproduction is entirely due to a specific increase in the relative rate of folate reductase synthesis by the resistant cells (1). We have presented evidence in this report that the high level of folate reductase synthesis is largely, and probably entirely, due to a corresponding increase in the level of translatable folate reductase mRNA. Control experiments indicated that the low level of folate reductase mRNA activity in cytoplasmic RNA from sensitive cells did not result from the presence of an inhibitor of in vitro folate reductase synthesis or a potent ribonuclease activity that destroyed folate reductase mRNA activity. While this manuscript was in preparation, Chang and Littlefield reported the use of somewhat different techniques to provide evidence for an increase in the level of folate reductase mRNA activity in methotrexate-resistant baby hamster kidney cells (15).

We have compared the rates of folate reductase synthesis with the levels of folate reductase mRNA activity under a variety of conditions known to affect the rate of folate reductase synthesis. These include not only (a) sensitive and methotrexate-resistant cells, but also (b) two lines of resistant cells having different rates of folate reductase synthesis, (c) growth of resistant cells in the absence of methotrexate, and (d) growth phase. The results of these independent determinations are compared in Fig. 1 where the relative rates of folate reductase synthesis in cells are plotted as a function of folate reductase mRNA activity assayed in the reticulocyte translation assay. The data clearly indicate that the major, and perhaps only, parameter governing the rate of folate reductase synthesis in each case is the level of translatable folate reductase mRNA.

The high levels of folate reductase mRNA activity present in the methotrexate-resistant cells, and the regulation of folate reductase mRNA activity by the factors mentioned above, presumably result from alterations in either the structure or metabolism of folate reductase mRNA. Such alterations may include those affecting the synthesis, degradation, utilization, or processing (including nuclear transport) of the message. In order to elucidate the role of these processes in the regulation of folate reductase mRNA activity, it will not only be necessary to have a reliable assay for the biological activity of the mRNA but, in addition, to have a specific sequence probe, i.e. a DNA complementary to folate reductase mRNA. These studies require the ability to prepare adequate quantities of folate reductase mRNA in pure form. We have therefore begun an initial characterization and partial purification of folate reductase mRNA.

Sedimentation analysis of folate reductase mRNA activity on isokinetic sucrose gradients (13) containing sodium dodecyl sulfate revealed a sedimentation coefficient of approximately 14 S. In addition, comparison with standards of known molecular weight (16) under conditions expected to eliminate most secondary structure (i.e. 85% formamide) suggested a molecular weight for folate reductase mRNA in the range of 4 x 10^6, a value consistent with that derived from equations relating sedimentation coefficient to molecular weight (17, 18).

This corresponds to about 1,200 nucleotides and is therefore somewhat larger than required to code for folate reductase (M_r = 21,000). However, in view of the uncertainties involved with relating sedimentation behavior to molecular weight these values must be considered tentative until more reliable molecular weight determinations are made. The fact that the molecular weight of the in vitro synthesized folate reductase was indistinguishable from that of authentic [3C]-labeled folate reductase suggests that extra nucleotides are not required to code for a higher molecular weight precursor, as observed for some secreted proteins (19-21). Some untranslated nucleotides are presumably accounted for by the presence of a poly(A) sequence on the mRNA (see below).

Poly(U)-Sepharose chromatography was used to separate folate reductase mRNA and other poly(A)-containing mRNAs (22) from rRNA. Sedimentation analysis of total poly(A)-containing mRNA showed that it was distributed over a broad range of sedimentation coefficients peaking in the region of 18 S. A similar size distribution of animal cell mRNAs has been observed by others (23, 24). The combined use of poly(U)-Sepharose and sucrose gradient fractionation procedures resulted in an approximately 90-fold purification of folate reductase mRNA activity over total cytoplasmic RNA. However, since folate reductase mRNA activity was not substantially separated from the bulk of the other mRNAs, and the sedimentation profile of poly(A)-containing mRNA revealed no peak of optical density in the region of folate reductase mRNA activity, fractionation of the mRNA on the basis of size will be of only marginal use as a step in its purification. Therefore, we are currently using the more specific indirect immunoprecipitation procedure previously employed in this laboratory for the purification of ovalbumin (25, 26), albumin (26), and conalbumin* mRNAs.

*For payvar, and K. T. Schimke, manuscript in preparation.
Acknowledgments—We are grateful to Evan Jones for assistance and advice during the initial phase of these investigations, Charissa Hogeboom for help with growing the cells, and Stephen Free for critical evaluation of the manuscript. Special thanks are extended to Marvin Wickens and Farhang Payvar for many helpful suggestions throughout the course of these investigations.

REFERENCES

1. Alt, F. W., Kellemes, R. E., and Schimke, R. T. (1976) J. Biol. Chem. 251, 3063–3074
2. Hakala, M. T., Zakrzewski, S. F., and Nichol, C. A. (1961) J. Biol. Chem. 236, 952–958
3. Zakrzewski, S. F., Hakala, M. T., and Nichol, C. A. (1966) Mol. Pharmacol. 2, 423–431
4. Rauno, R. P., and Hakala, M. T. (1967) Mol. Pharmacol. 3, 279–283
5. McKnight, G. S., and Schimke, R. T. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 4327–4331
6. Palmiter, R. D., Palacios, R., and Schimke, R. T. (1979) J Biol Chem. 247, 3296–3304
7. Evans, M. J., and Lingrel, J. B. (1969) Biochemistry 8, 829
8. Lockard, R. E., and Lingrel, J. B. (1969) Biochem. Biophys. Res. Commun. 37, 204
9. Schimke, R. T., Rhoads, R. E., and McKnight, G. S. (1974) Methods Enzymol. 30, 694–701
10. Haines, M. E., Carey, N. H., and Palmiter, R. D. (1974) Eur. J. Biochem. 43, 549–559
11. Holmes, D. S., and Bonner, J. (1973) Biochemistry 12, 2339–2338
12. Ojala, D., and Attardi, G. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 365–367
13. McCarty, K. S., Jr., Vollmer, R. T., and McCarty, K. S. (1974) Anal. Biochem. 61, 165–183
14. Maunoughton, M., Freeman, K. B., and Bishop, J. O. (1974) Cell 1, 117–125
15. Chang, S. E., and Littlefield, J. W. (1976) Cell 7, 391–396
16. McConkey, E. H., and Hopkins, J. W. (1969) J. Mol. Biol. 39, 545–550
17. Spirin, A. S. (1961) Biokhimiya 26, 511
18. Gierer, A. (1950) Z. Naturforsch. 13b, 477
19. Devillers-Thiery, A., Kindt, T., Schiefer, G., and Blobel, G. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 5016–5020
20. Kemper, B., Habener, J. F., Ernst, M. D., Potts, J. T., and Rich, A. (1976) Biochemistry 15, 15–19
21. Milstein, C., Brownlee, G. G., Harrison, T. M., and Mathews, M. B. (1972) Nature New Biol. 239, 117
22. Brawerman, G. (1974) Annu. Rev. Biochem. 43, 621–642
23. Singer, R. H., and Penman, S. (1973) J. Mol. Biol. 78, 321–334
24. Puckett, L., Chambers, S., and Darnell, J. E. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 389–393
25. Shapiro, D. J., and Schimke, R. T. (1975) J. Biol. Chem. 250, 1759–1764
26. Shapiro, D. J., Taylor, J. M., McKnight, G. S., Palacios, R., Gonzalez, C., Kiely, M. L., and Schimke, R. T. (1974) J. Biol. Chem. 249, 3665–3671
Regulation of folate reductase synthesis in sensitive and methotrexate-resistant sarcoma 180 cells. In vitro translation and characterization of folate reductase mRNA.
R E Kellems, F W Alt and R T Schimke

J. Biol. Chem. 1976, 251:6987-6993.

Access the most updated version of this article at http://www.jbc.org/content/251/22/6987

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/251/22/6987.full.html#ref-list-1