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Sensitivity of a Mutator Gene in Chinese Hamster Ovary Cells to Deoxynucleoside Triphosphate Pool Alterations

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The Thy⁻ mutants of Chinese hamster ovary cells have a 5- to 10-fold elevated pool of deoxycytidine 5'-triphosphate (dCTP) and are auxotrophic for thymidine as an apparent consequence of a single mutation. *thy* is also a mutator gene, elevating the spontaneous rate of mutation 5- to 200-fold for at least two genetic markers. Previous experiments suggested that this mutator activity was caused by the elevated pool of dCTP in Thy⁻ cells. To test this, the dCTP and deoxythymidine 5'-triphosphate (dTTP) pools were manipulated by altering the external concentration of thymidine in the growth medium. The rate of mutation at one genetic locus, ouabain resistance, was directly related to cellular dCTP content. At the highest level of dCTP the rate in one Thy⁻ strain was ~200 times that of wild-type cells. However, the relationship between dCTP content and the rate of mutation at the ouabain locus was different for two mutator strains and wild-type cells. The rate of mutation at a second locus, thioguanine resistance, was increased ~10-fold over wild type regardless of the dCTP-dTTP pools. These experiments suggest that the mutator activity of *thy* is clearly related to dCTP content, but the dCTP level alone does not appear to be the cause of the mutator.

Cells require a continuous and balanced supply of deoxynucleoside triphosphates for replication of deoxyribonucleic acid (DNA) (28). Pools of deoxynucleoside triphosphate accumulate in cells but are very small and are sufficient to sustain DNA synthesis for only a short time (26, 30). Recently, evidence has been presented that the enzymes which synthesize deoxynucleoside triphosphates are organized into a complex which "channels" the supply of these compounds to the replication fork from the ribonucleoside diphosphates, suggesting that the pools themselves may not function as the direct precursors of DNA synthesis (27). Nevertheless there is good evidence that the pools play a key role in the control of the supply of deoxynucleoside triphosphates through feedback inhibition of the enzyme ribonucleotide reductase (3, 14, 22, 25).

We have previously described the isolation of a class of arabinosylcytosine- and thymidine-resistant Chinese hamster ovary (CHO) cell mutants that also require thymidine for growth (*thy*; 24). This class of mutants has a 5- to 10-fold expanded pool of deoxycytidine 5'-triphosphate (dCTP), but it is also dependent upon an exogenous source of thymidine for maintenance of the deoxythymidine 5'-triphosphate (dTTP) pool. The thymidine requirement of Thy⁻ cells can be satisfied by deoxyuridine or deoxycytidine but not by the other ribo- or deoxyribonucleosides. Thy⁻ revertants, selected by their ability to grow in the absence of added thymidine, become sensitive to arabinosylcytosine and in most cases thymidine (24) due to the restoration of the dCTP pool to near normal levels (23). In somatic cell hybrids between Thy⁻ mutants and wild-type cells, resistance to arabinosylcytosine is dominant or codominant, whereas the thymidine requirement is recessive. The dCTP level of these hybrids is intermediate between wild-type and Thy⁻ mutants. On the basis of these data we suggested that *thy* is a single mutation of ribonucleotide reductase. However, experiments with cell-free preparations of reductase have not shown any difference in the level of reductase or in the sensitivity of the enzyme to effector molecules (using cytidine, uridine, or guanosine diphosphates as a substrate). In contrast, another class of arabinosylcytosine-resistant mutants, which differ from Thy⁻ mutants in that they are resistant to deoxyadenosine and prototrophic for thymidine (24), have demonstrable alterations of ribonucleoside diphosphate reductase activity. We have also examined the level of two other enzymes involved in the synthesis of dTTP, deoxyuridine triphosphate hydrolase and deoxycytidylate deaminase. The levels of both these enzymes were also unchanged in Thy⁻ strains relative to wild type

(both Thy⁻ and wild-type cell strains were deficient in the deaminase).

The property of *thy* that has attracted our attention is its mutator activity (23). Like the excess dCTP pool, the mutator is codominant in hybrid cells, and initial experiments suggested that the increased rate of mutation in these lines was related to the altered balance of pools of deoxynucleoside triphosphates caused by the excess pool of dCTP.

In the experiments reported here, intracellular concentrations of deoxynucleoside triphosphates were manipulated by altering the external thymidine level of both Thy⁻ mutants and the parental strain. The effect of these manipulations on the rate of mutation at three genetic loci was determined. Here I present evidence of a clear relationship between pools of dCTP and the rate of mutation at two genetic loci in mutant and, to some extent, wild-type cell strains. However, the evidence suggests that the pool alterations alone are not the cause of the high mutational rate of Thy⁻ strains.

MATERIALS AND METHODS

Materials. Powdered medium and fetal calf serum for cell culture were purchased from GIBCO Laboratories, Grand Island, N.Y. Aminopterin, hypoxanthine, 6-thioguanine, ouabain, and emetine were purchased from Sigma Chemical Co., St. Louis, Mo. Thymidine and deoxynucleoside triphosphates were products of P-L Biochemicals, Milwaukee, Wis. Plastic dishes for cell culture were obtained from NUNC Products (Roskilde, Denmark) and Costar (Cambridge Mass.).

Cell lines and culture techniques. The derivation of the cell strains used in these experiments has been described in previous communications (23, 24). The wild-type strain was the CHO proline-requiring cell line obtained from Louis Siminovitch. Thy⁻49 and 303 are independent thymidine-requiring mutant strains obtained by selection in arabinosylcytosine and thymidine. Cells were maintained in suspension culture at 37°C in alpha minimal essential medium (GIBCO) plus 8% fetal calf serum (GIBCO). Thymidine (10 μM for wild type and 100 μM for Thy⁻49 and 303), but not the other ribo- or deoxyribonucleosides, was added to all cultures. During serial cultivation, cultures were diluted to 2×10^4 cells per ml and then allowed to grow to about 6×10^5 cells per ml before redilution. All cell strains have been periodically checked for mycoplasma contamination by staining with Hoechst dye (10) and were found to be free of contamination.

In experiments in which it was necessary to precisely control the exogenous thymidine concentration, cells were cultured in alpha minimal essential medium with 8% dialyzed fetal calf serum and the desired thymidine concentration. Fetal calf serum was dialyzed against a 10-fold excess of phosphate-buffered saline solution over 4 days with four changes of the solution. The serum was then filtered through a 0.22-μm membrane filter with an AP15 prefilter (Millipore Corp., Bedford, Mass.).

Genetic markers. To determine the rates of mutation in our strains, three genetic markers independent of the Thy⁻ phenotype were used: 6-thioguanine resistance (*thg*^{r}), ouabain resistance (*oua*^{R}), and emetine resistance (*emt*^{r}). 6-Thioguanine-resistant (6-tg^r) mutants have been shown to be deficient or altered in hypoxanthine guanine phosphoribosyltransferase (HPRT⁻) by previous investigators (8). For determination of 6-tg^r mutants, cultures were plated in medium containing 10 μM 6-thioguanine, 10 μM thymidine, and 8% dialyzed fetal calf serum. Plating densities were not greater than 5×10^5 per 100-mm dish. Reconstruction experiments indicated that this cell density did not reduce the frequency of *thg*^{r} mutations in our cultures. Since HPRT⁻ cells are unable to grow in HAT medium (31), we also tested a number of our 6-tg^r mutants in HAT medium consisting of 10^{-4} M hypoxanthine, 10^{-6} M aminopterin, and 10^{-5} M thymidine.

Ouabain is an inhibitor of the Na^+-K^+ transport system of mammalian cells, and cells resistant to this drug have an Na^+-K^+ adenosine triphosphatase more resistant to ouabain (2). To determine the number of ouabain-resistant mutants in a culture, up to 10^6 cells were plated per 100-mm dish in 2 mM ouabain in alpha minimal essential medium with 8% dialyzed fetal calf serum and 10 μM thymidine.

Emetine is a potent protein synthesis inhibitor in mammalian cells (17). CHO cells resistant to this drug have alterations of the 40S ribosomal subunit, rendering protein synthesis, as measured in vitro, resistant to inhibition by emetine (4, 17, 18). A sample of 2×10^6 cells per 100-mm dish was plated in 0.2 μM emetine in the experiments to determine the number of emetine-resistant cells.

In all these experiments, cells were incubated for 7 days in the selective medium, and then plates were fixed and stained with 0.5% methylene blue in 50% methanol. Only colonies with more than 50 cells were counted. Occasionally drug-resistant clones were taken and serially cultivated in nonselective medium for further screening. The drug resistance phenotype was stable in all cases.

Determination of mutational rates. In these experiments, mutational events have been quantified by measuring the rate of mutation by using the Luria-Delbrück (20) fluctuation test. Mutation frequencies for our strains vary considerably because of the high background of resistant cells in Thy⁻ strains. For each fluctuation experiment 15 to 30 replicate cultures of each cell line were grown in alpha minimal essential medium supplemented with 8% dialyzed fetal calf serum and the desired thymidine concentration from an inoculation density of 100 cells per replicate culture. When these cultures had reached the desired density, the entire cell population was plated in the appropriate selective medium. Mutational rates were calculated from the fraction of cultures containing no mutants. It was necessary to use this calculation since one of the mutants, Thy⁻49, has an unusually rounded morphology and only loosely attaches to plastic culture dishes. It has also been suggested that 6-tg^r, resistant HPRT⁻ cells grow more rapidly than sensitive cells in medium with excess thymidine (6). By calculating the mutational rate on the basis of cultures with no mutants, we have avoided this complication.

Deoxynucleoside triphosphate pool measurements. Deoxynucleoside triphosphates were extracted from cell monolayers growing in medium with defined thymidine concentrations by using ice-cold 60% methanol. Measurement was made by high-performance liquid chromatography using a Whatman Partisil-10-SAX column, with 0.30 M ammonium phosphate (pH 3.45) at 1.0 or 1.5 ml/min as the mobile phase (16). Elutions were monitored at 254 nm with a Waters variable-wavelength detector. Because the excess of ribonucleotides in the cells obscures the deoxyribonucleotides, it was necessary to degrade ribonucleotides by using sodium periodate (16, 27). Even with this digestion it was not always possible to measure deoxyguanosine 5'-triphosphate (dGTP) pools in the CHO cell strains. For some measurements it was therefore necessary to use the more sensitive technique (30) of the defined copolymer polydeoxyinosinate-polydeoxycytidylate and DNA polymerase I (both from Boehringer-Mannheim, Montreal, Canada). At least two determinations were made for each culture, and two to three independent experiments were performed for each thymidine concentration. The pool values were normalized to the amount of DNA measured in the methanol precipitate (3).

RESULTS

Manipulation of deoxynucleoside triphosphate pools in wild-type and Thy$^-$ cell lines. The pools of deoxynucleoside triphosphates were altered by changing the external thymidine concentration over a range that did not greatly affect cellular viability or growth. Wild-type cells were grown either in the absence of thymidine or in concentrations as high as 100 μM. Thy$^-$ mutants were grown in concentrations of thymidine ranging from 1 to 2,000 μM. Changing the thymidine concentration allowed manipulation to some extent of dTTP, dCTP, and dGTP pools (Fig. 1). These manipulations were possible because of the regulatory effects of dTTP on the reduction of both cytidine diphosphate and guanosine diphosphate by the enzyme ribonucleoside diphosphate reductase (14, 25). dTTP is a negative effector of cytidine diphosphate reduction, so increased intracellular concentrations of dTTP, produced by increasing the exogenous thymidine concentrations, decreased the pool of dCTP. Thy$^-$ mutants are considerably more resistant to growth inhibition by thymidine as a result of the expanded pool of dCTP. Consequently, these cells were able to tolerate a greater pool of dTTP before growth was inhibited due to lack of dCTP. dTTP is an essential positive effector of guanosine diphosphate reduction (14, 25), and thymidine starvation of Thy$^-$ mutants leads to depletion of both dTTP and dGTP pools (24). In these experiments, varying the concentration of thymidine in the medium of wild-type and Thy$^-$ strains led to parallel changes of dTTP and dGTP. To alter the pattern of these parallel pool changes, deoxyguanosine was added to the medium of wild type and Thy$^-$49. However, the added deoxyguanosine did not change the pool of dGTP. Most likely this was due to the catabolism of deoxyguanosine (9) and perhaps an inhibition of phosphorylation of deoxyguanosine by the high intracellular dCTP. High dCTP also appears to inhibit phosphorylation of deoxyadenosine to deoxyadenosine 5'-triphosphate (22).

Dependence of the rate of mutation to Oua^R on the deoxynucleoside triphosphate content of wild-type and Thy$^-$ cell strains. The effect of alterations of thymidine concentrations on the rate of mutation to ouabain resistance is presented in Fig. 2. For wild type, the highest rate of mutation was obtained in the absence or in low concentrations of thymidine. Increasing concentrations of thymidine caused the rate of mutation first to decrease, to reach its lowest point at 20 μM, and then to increase with increasing thymidine. The difference between the highest and lowest rates was 10-fold. Thy$^-$ mutants showed a similar, though greater, response in mutational rate to alterations of thymidine concentration. The highest rate of mutation was again observed in low concentrations of thymidine (1 μM), and the minima were observed in 100 μM for Thy$^-$49 and about 400 μM thymidine for Thy$^-$303. Increasing the concentration of thymidine above these levels produced an increase in the rate of mutation. The Thy$^-$ mutants were much more sensitive than wild-type cells to changes in thymidine concentration: the greatest difference in mutation rate was 16-fold for Thy$^-$49 and 250-fold for Thy$^-$303. The rate of mutation to Oua^R for the Thy$^-$ mutants was greater than that of wild type at all concentrations of thymidine. The difference varied with thymidine concentration and was greatest (160-fold) between 4 and 10 μM.

In Fig. 3 the log of mutational rates obtained at the different thymidine concentrations has been plotted against the log of deoxynucleoside triphosphate content of the cells at the same concentrations (see Fig. 1). In the presence of low thymidine (Fig. 3A), there was a clear relationship between the rate of mutation to Oua^R and the pool of dCTP for Thy$^-$49 and 303 although the slope was different for the two cell strains. High levels of dCTP also had an effect on wild-type cells, but no clear single relationship emerged. Plotting the mutational rates obtained in low thymidine against dTTP content did not produce a clear response. In the presence of high thymidine (Fig. 3B) the rate of mutation to Oua^R was dependent upon the level of dTTP for all three cell strains. It is clear that the mutational rate in high thymidine was not de-

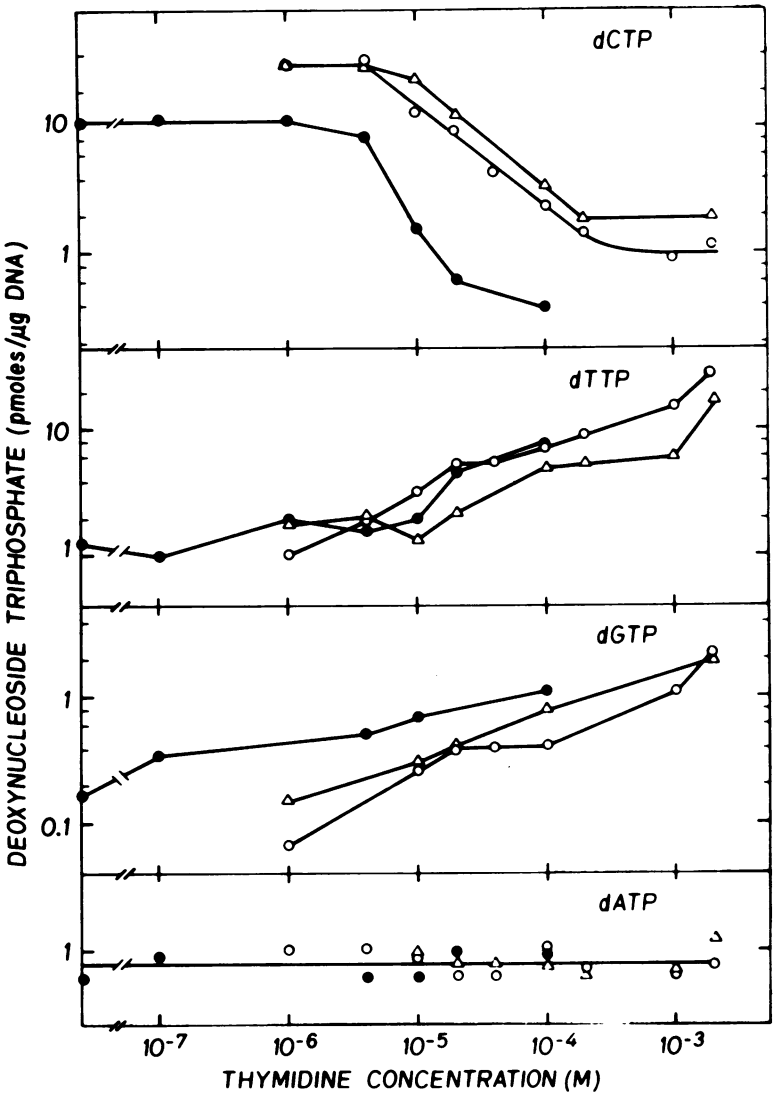

FIG. 1. *Effect of exogenous thymidine on the deoxynucleoside triphosphate content of wild-type and Thy⁻ cell lines. Cells were grown in defined concentrations of thymidine for 3 days before harvesting and analysis of pool content as described in the text. Each point represents the average of at least two independent experiments. Wild type* (●); *Thy⁻49* (○); *Thy⁻303* (△).

pendent upon dCTP concentration since conditions producing very similar dCTP levels produced very different mutational rates.

Effect of deoxynucleoside triphosphate pool alterations on the rate of mutation to 6-tg^r. Altering the thymidine concentration of the medium also altered the rate of mutation to 6-tg^r (Fig. 4). As before, low concentrations of thymidine produced the highest mutational rate. However, the response of mutational rate at this locus (*thg*) was less dramatic than that at *oua*, as the variation in mutational rate for all cell strains was only about fivefold. The greatest difference in the rate of mutation to 6-tg^r between wild-type and Thy⁻ strains was 12-fold at 40 μM thymidine. High concentrations of thymidine also increased the rate of mutation of the Thy⁻ strains to 6-tg^r but did not significantly affect wild type. Administration of lethal concentrations of thymidine to wild-type cells (10 mM thymidine for 24 h), however, did increase the frequency of 6-thioguanine-resistant mutants (unpublished data).

In Fig. 5 the log of the rate of mutation to 6-

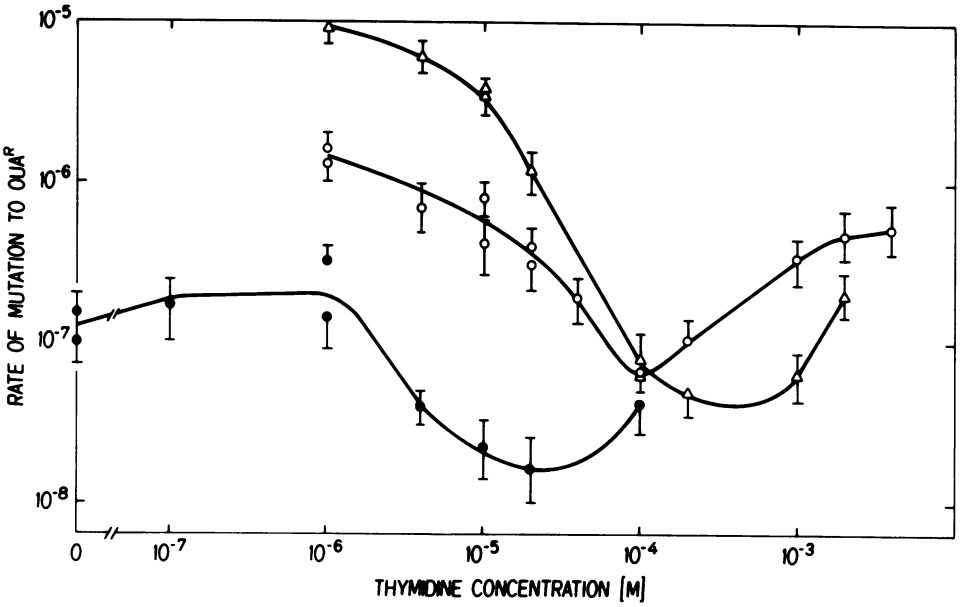

FIG. 2. *Effect of exogenous thymidine on the rate of mutation to Oua^R. Replica cultures of wild type (●), Thy$^-$49 (○), or Thy$^-$303 (△) were grown in medium containing defined concentrations of thymidine. At the appropriate cell density the entire culture was plated in medium containing ouabain to determine the fraction of cultures containing Oua^R mutants. The mutational rate to Oua^R was determined on the basis of the fraction of cultures containing no mutants, termed the P_0 calculation (20).*

tg^r at low thymidine concentrations has been plotted as a function of the log of the dCTP or dTTP content. The rate of mutation for this marker in wild type did not show a strong dependence on dCTP content, increasing only slightly with increased intracellular levels of this deoxynucleoside triphosphate. The rate appeared to show a more pronounced inverse relationship to dTTP concentration. For Thy$^-$49 and 303 the rate of mutation appeared to be directly related to dCTP content and inversely to dTTP. For this marker, the two Thy$^-$ mutants showed similar responses to dCTP and dTTP.

Effect of deoxynucleoside triphosphate pools on *emt* locus. Previous experiments had suggested that the *thy* mutator gene is site specific and does not act upon the *emt* locus (23). In these experiments I have shown that the mutational rate of Thy$^-$ cells can be stimulated by growing cells in low thymidine. To determine whether low thymidine could also stimulate the rate of mutation to Emt^r, wild-type and Thy$^-$ strains were grown in a range of thymidine concentrations including those which produced maximum mutagenesis for the *oua* and *thg* markers. The results of these experiments are presented in Table 1. There was no significant difference in mutational rates to Emt^r of cells grown in low thymidine relative to those grown in 100 μM thymidine. Excess thymidine caused a small but significant increase in mutational rate to Emt^r for Thy$^-$49 and 303.

DISCUSSION

In these experiments I have demonstrated that manipulations of the pools of dCTP and dTTP produce clear changes in the rates of mutation to both 6-tg^r and Oua^R in wild-type and Thy$^-$ strains.

Even though the mutator activity of *thy* is strongly affected by the pools of deoxynucleoside triphosphates, the pool imbalance alone does not appear to be the cause of the higher rate of mutation in Thy$^-$ strains. If mutational rates were dependent upon the dCTP content and a Thy$^-$ strain had a greater rate of mutation simply because of its higher pool of dCTP, then one would predict a common relationship between dCTP content and mutational rate for both mutator and wild-type strains. Such a relationship for the rate of mutation to Oua^R and the dTTP content is evident in high thymidine concentrations (Fig. 3B). I have also demonstrated that sensitivity to DNA alkylating agents is a function of dCTP/dTTP ratio for all three strains (21). However, in the presence of low thymidine the relationships of mutational rate to pool content are clearly different. Very similar levels of dCTP in the three strains produce very different mutational rates.

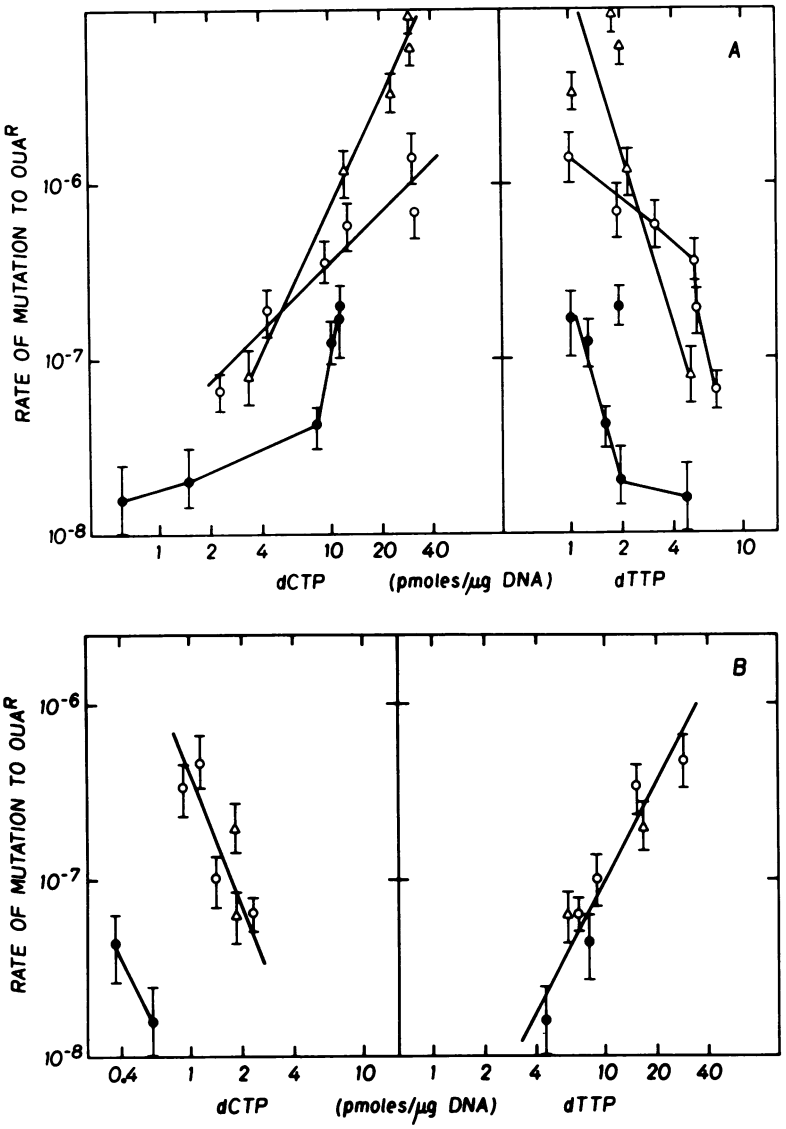

FIG. 3. *Rate of mutation to Oua^R as a function of dCTP or dTTP content. Data from Fig. 1 and 2 were used for this figure. The relationships of pool content and mutational rate in low thymidine (A) and high thymidine (B) are presented. Low and high thymidine are defined on the basis of the data in Fig. 2. At one thymidine concentration for each cell strain, the mutational rate to Oua^R reached a minimum level. Thymidine concentrations lower than this point are defined as low thymidine. Thymidine concentrations greater than this are high thymidine. Wild type (●); Thy⁻49 (○); Thy⁻303 (△).*

The responses of the three markers *oua*^{R}, *thg*^{r}, and *emt*^{r} to changes in the pool content were markedly different. The rate of mutation to Oua^R showed extreme sensitivity to changes in pool level, whereas that to 6-tg^r showed relatively little variation. This could be due to a difference in the types of mutations which can be detected by the two markers. It has been suggested that Oua^R is the result of only base pair changes (1), whereas the loss of hypoxanthine guanine phosphoribosyltransferase activity in 6-tg^r cells could be due to base pair changes, deletions, insertions, or frameshifts (8). The results presented here would then suggest that the *thy* mutator gene is very proficient at producing base pair mutations, since the rate of mutation to Oua^R is so strongly affected. The data also suggest that *thy* may increase the

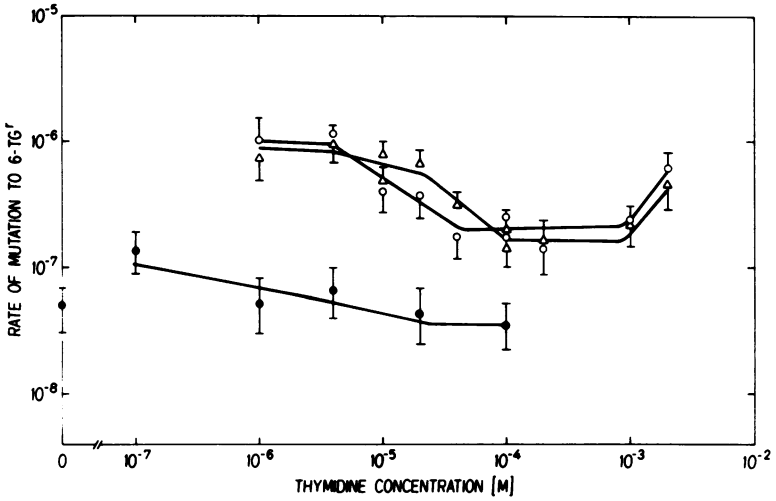

FIG. 4. *Effect of exogenous thymidine on the rate of mutation to 6-tg^r. Replica cultures of wild type (●), Thy$^-$49 (○), or Thy$^-$303 (△) were grown in medium containing defined concentrations of thymidine. At the appropriate cell density, the entire culture was plated in medium containing 6-thioguanine to determine the fraction of cultures containing 6-tg^r mutants. The mutational rate to 6-tg^r was determined on the basis of a P_0 calculation.*

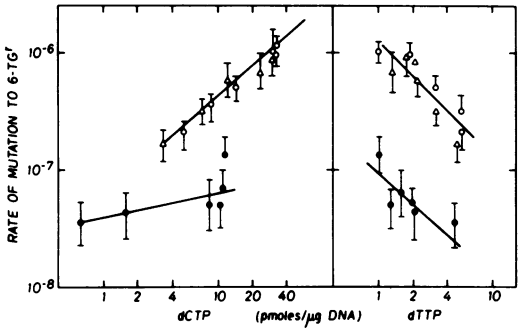

FIG. 5. *Rate of mutation to 6-tg^r as a function of dCTP or dTTP content. Data from Fig. 1 and 4 were used for this figure. The relationship of pool content and 6-tg^r in low thymidine (<20 μM for Pro$^-$ and <100 μM for the Thy$^-$ strains) only is presented here since there were too few points to make a significant plot in higher concentrations of thymidine. Wild type (●); Thy$^-$49 (○); Thy$^-$303 (△).*

occurrence of other types of mutations, as the rate of mutation to 6-tg^r is uniformly higher than that in wild type in all conditions. However, it is difficult to substantiate these suggestions in the absence of specific probes for characterization of genetic damage at these loci.

The rate of mutation to Emt^r was unaffected by *thy* at concentrations of thymidine which produced maximum rates of mutation to Oua^R or 6-tg^r. The explanation for this is not clear. We are examining the rate of mutation at a locus closely linked to *emt*, chromate resistance (7), to determine whether the site specificity extends over a significant part of chromosome 2 of CHO.

The increase of mutational rate caused by excess thymidine (5) appears to proceed by a different pathway from that caused by *thy*. The rate of mutation to both Oua^R and 6-tg^r in wild-type and Thy$^-$ cells is stimulated by excess (sublethal) thymidine. This mutagenesis is directly related to the pool of dTTP, and values for all cell strains give a common relationship. This suggests that excess thymidine has a greater effect on Thy$^-$ mutants simply because they can tolerate more dTTP. Furthermore, excess thymidine causes a small but significant increase in the rate of mutation at the *emt* locus, whereas *thy* does not.

mutD, a mutator gene of *Escherichia coli*, shows a dependence on exogenous thymidine similar to that demonstrated for *thy* (12, 13). Evidence suggests that *mutD* may be a protein active in DNA replication, since two suppressors of the mutator activity map in the *nalA* gene (12). Thus there appear to be proteins important for the faithful replication of DNA which are sensitive to deoxynucleoside triphosphate fluctuations. In vitro experiments with reconstructed bacteriophage systems suggest that both base pairing and proofreading are also dependent upon deoxynucleoside triphosphate levels (11, 15, 19). It is possible that the *thy* mutator gene is an alteration of a replication protein conferring increased sensitivity to deoxynucleoside triphosphate variations. Furthermore, the increased level of dCTP in these cells could be a consequence of misincorporation of

TABLE 1. *Effect of exogenous thymidine on the rate of mutation to emetine resistance*

Cell line	Mutation rate in thymidine concn:		
	Low[a]	100 μM	2,000 μM
Wild type	$1.4 \pm 0.6 \times 10^{-8}$	$1.8 \pm 0.8 \times 10^{-8}$	
Thy⁻49	$3.4 \pm 1.3 \times 10^{-8}$	$1.7 \pm 0.8 \times 10^{-8}$	$6.6 \pm 1.5 \times 10^{-8}$
Thy⁻303	$4.8 \pm 3.2 \times 10^{-9}$	$2.1 \pm 1.1 \times 10^{-8}$	$4.3 \pm 2.0 \times 10^{-8}$

[a] Low thymidine is medium without thymidine for wild type and 4 μM for Thy⁻49 and 303. Replica cultures were grown in medium containing the specified thymidine concentration and then examined for the presence of *emt*^{r} mutations as described in the text. Mutational rates were determined by a P_0 calculation.

dCTP followed by excision by the normal proofreading mechanisms, rather than a cause of the misincorporation. Although the evidence presented here is consistent with such an explanation there is no direct evidence for it. It is equally possible that the differences observed between mutational rate and pool content are due to subtleties of intracellular deoxynucleoside triphosphate localization in mutant and wild-type strains. Thus, though these studies provide evidence for a role of deoxynucleoside triphosphate pools in maintaining the fidelity of DNA replication, the mechanism by which they fulfill this role and how it is altered by the *thy* mutation remain unresolved.

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LITERATURE CITED

1. **Baker, R. M.** 1979. Nature and use of ouabain-resistant mutants. Banbury Rep. **2**:237–247.
2. **Baker, R. M., D. M. Brunette, R. Mankovitz, L. H. Thompson, G. F. Whitmore, L. Siminovitch, and J. E. Till.** 1974. Ouabain-resistant mutants of mouse and hamster cells in culture. Cell **1**:9–21.
3. **Bjursell, G., and P. Reichard.** 1973. Effects of thymidine on deoxyribonucleoside triphosphate pools and deoxyribonucleic acid synthesis in Chinese hamster ovary cells. J. Biol. Chem. **248**:3904–3909.
4. **Boersma, D., S. M. McGill, J. W. Mollenkamp, and D. J. Roufa.** 1979. Emetine resistance in Chinese hamster cells is linked genetically with an altered 40S ribosomal subunit protein, S20. Proc. Natl. Acad. Sci. U.S.A. **76**: 415–419.
5. **Bradley, M. O., and N. A. Sharkey.** 1978. Mutagenicity of thymidine to cultured Chinese hamster cells. Nature (London) **274**:607–608.
6. **Brennand, J., and M. Fox.** 1980. Excess thymidine is not mutagenic in Chinese hamster V_{79} fibroblasts. Cell Biol. Int. Rep. **4**:923–932.
7. **Campbell, C. E., and R. G. Worton.** 1980. Linkage of genetic markers *emt* and *chr* in Chinese hamster cells. Somatic Cell Genet. **6**:215–224.
8. **Caskey, C. T., and G. D. Kruh.** 1979. The HPRT locus. Cell **16**:1–9.
9. **Chan, T.-S.** 1978. Deoxyguanosine toxicity on lymphoid cells as a cause for immunosuppression in purine nucleoside phosphorylase deficiency. Cell **14**:523–530.
10. **Chen, T. R.** 1977. In situ detection of mycoplasma contamination in cell cultures by fluorescent Hoechst 33258 stain. Exp. Cell Res. **104**:255–262.
11. **Clayton, L. K., M. F. Goodman, E. W. Branscomb, and D. J. Galas.** 1979. Error induction and correction by mutant and wild type T4 DNA polymerases. J. Biol. Chem. **254**:1902–1912.
12. **Cox, E. C.** 1976. Bacterial mutator genes and the control of spontaneous mutation. Annu. Rev. Genet. **10**:135–156.
13. **Degnen, G. E., and E. C. Cox.** 1974. Conditional mutator gene in *Escherichia coli*: isolation, mapping, and effector studies. J. Bacteriol. **117**:477–487.
14. **Eriksson, S., L. Thelander, and M. Åkerman.** 1979. Allosteric regulation of calf thymus ribonucleoside diphosphate reductase. Biochemistry **18**:2948–2952.
15. **Fersht, A. R.** 1979. Fidelity of replication of phage φX174 DNA by DNA polymerase III holoenzyme: spontaneous mutation by misincorporation. Proc. Natl. Acad. Sci. U.S.A. **76**:4946–4950.
16. **Garrett, C., and D. V. Santi.** 1979. A rapid and sensitive high pressure liquid chromatography assay for deoxyribonucleoside triphosphates in cell extracts. Anal. Biochem. **99**:268–273.
17. **Gupta, R. S., and L. Siminovitch.** 1976. The isolation and preliminary characterization of somatic cell mutants resistant to the protein synthesis inhibitor—emetine. Cell **9**:213–219.
18. **Gupta, R. S., and L. Siminovitch.** 1977. The molecular basis of emetine resistance in Chinese hamster ovary cells: alteration in the 40S ribosomal subunit. Cell **10**: 61–66.
19. **Hibner, U., and B. M. Alberts.** 1980. Fidelity of DNA replication catalysed *in vitro* on a natural DNA template by the T4 bacteriophage multi-enzyme complex. Nature (London) **285**:300–305.
20. **Luria, S. E., and M. Delbrück.** 1943. Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28**:491–511.
21. **Meuth, M.** 1981. Role of deoxynucleoside triphosphate pools in the cytotoxic and mutagenic effects of DNA alkylating agents. Somatic Cell Genet. **7**:89–102.
22. **Meuth, M., E. Aufreiter, and P. Reichard.** 1976. Deoxyribonucleotide pools in mouse-fibroblast cell lines with altered ribonucleotide reductase. Eur. J. Biochem. **71**:39–43.
23. **Meuth, M., N. L'Heureux-Huard, and M. Trudel.** 1979. Characterization of a mutator gene in Chinese hamster ovary cells. Proc. Natl. Acad. Sci. U.S.A. **76**:6505–6509.
24. **Meuth, M., M. Trudel, and L. Siminovitch.** 1979. Selection of Chinese hamster cells auxotrophic for thymidine by 1-β-D-arabinofuranosyl cytosine. Somatic Cell Genet. **5**:303–318.
25. **Moore, E. C., and R. B. Hurlbert.** 1966. Regulation of mammalian deoxyribonucleotide biosynthesis by nucleotides as activators and inhibitors. J. Biol. Chem. **25**: 4802–4809.
26. **Nordenskjöld, B. A., L. Skoog, N. C. Brown, and P. Reichard.** 1970. Deoxyribonucleotide pools and deoxyribonucleic acid synthesis in cultured mouse embryo

cells. J. Biol. Chem. **245**:5360–5368.
27. **Reddy, G. P. V., and A. B. Pardee.** 1980. Multienzyme complex for metabolic channeling in mammalian DNA replication. Proc. Natl. Acad. Sci. U.S.A. **77**:3312–3316.
28. **Reichard, P.** 1978. From deoxynucleotides to DNA synthesis. Fed. Proc. **37**:9–14.
29. **Schmidt, G.** 1968. Periodate oxidation of ribonucleic acids and their derivatives. Methods Enzymol. **12B**:230–235.
30. **Skoog, L., and B. Nordenskjöld.** 1971. Effects of hydroxyurea and 1-β-D-arabinofuranosyl cytosine on deoxyribonucleotide pools in mouse embryo cells. Eur. J. Biochem. **19**:81–89.
31. **Szybalski, W., E. H. Szybalska, and G. Ragni.** 1962. Genetic studies with human cell lines. *In* R. E. Stevenson (ed.), Syverton Memorial Symposium on Analytical Cell Culture. Natl. Cancer Inst. Monogr. **7**:75–89.