Mutator phenotypes in mammalian cell mutants with distinct biochemical defects and abnormal deoxyribonucleoside triphosphate pools

(mutagenesis/ribonucleotide reductase/dCMP deaminase/S-49 T-lymphosarcoma)

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ABSTRACT Recent studies of in vitro DNA synthesis have shown that the fidelity of replication is influenced by the relative concentrations of deoxyribonucleoside triphosphates (dNTPs). Several investigators have used reconstituted prokaryotic replication systems to copy defined natural templates and have shown that specific incorporation errors can be induced by an appropriate bias of the precursor pools. The recent demonstration of mutator phenotypes among mutant Chinese hamster ovary cell lines with altered intracellular dNTP pools has allowed extension of the in vitro observations to eukaryotic replication and repair mechanisms. We describe here three mutant murine T-lymphosarcoma cell lines with altered dNTP pools and increased rates of spontaneous mutation to dexamethasone resistance and 6-thioguanine resistance. Unlike previously described mammalian cells with mutator phenotypes, these three lines have demonstrable defects in known structural gene products. Two of these cell lines are heterozygous for mutations affecting the M1 subunit of ribonucleoside diphosphate reductase; the other mutant is deficient in deoxyctytidylate deaminase. In each cell line these mutations result in decreased endogenous dNTP pools and increased rates of spontaneous mutation, which are shown to be characteristic of the cell line and independent of the two genetic markers examined. Furthermore, normalization of the dNTP pools of the deaminase-deficient cells suppresses its mutator phenotype. Thus, abnormal dNTP pools seem to cause enhanced mutagenesis in mammalian cells.

The degree of error in DNA replication and repair systems contributes to the characteristic mutation rate of any organism and therefore influences the counterpoise of species preservation and evolution. In vitro reconstitution of prokaryotic DNA synthetic machinery has allowed dissection of the determinants of replicative fidelity, and recent studies using natural templates of known nucleotide sequences have stressed the importance of relative deoxyribonucleoside triphosphate (dNTP) concentrations in mediating base substitution and misincorporation (1–3). Using amber mutants of E. coli DNA polymerase III, Fersht (2) measured reversion frequency in the product DNA by a transfection assay. He found that the rates of occurrence for specific incorporation errors obeyed a simple kinetic expression that included, as independent variables, the concentrations of the dNTPs competing for the site of potential reversion and the concentration of the next nucleotide to be added. Hibner and Alberts (3) analyzed reversion among dX174 amber mutants by using a system composed of seven purified T4 replication proteins and observed a linear relationship between the probability of a misincorporation of a nucleotide and the concentration ratios of incorrect to correct dNTP substrate. In the latter experiments, the fidelity of replication and frequency of contaminant wild-type virus were so high that reversion induction could be detected only in a context of biased precursor pools strongly favoring the necessary specific nucleotide transition.

Meuth et al. (4, 5) recently reported the isolation of three variant mammalian cell lines with increased intracellular dCTP concentrations and thymidine auxotrophy (thy+); however, the identity of the defective gene(s) product responsible for this phenotype is unknown. Each of these cell lines possesses an increased frequency of spontaneous reversion to thymidine prototrophy and an increased rate of spontaneous mutation to 6-thioguanine resistance or ouabain resistance when compared to control cells without the thy+ mutation (5). Perturbations of intracellular dNTP concentrations may also mediate the 5-bromodeoxyuridine- and thymidine-induced mutagenesis characteristic of specific mammalian cell systems (6–8). Thus, the effects of precurspool bias on the fidelity of in vitro prokaryotic DNA replication may also apply to DNA replication in eukaryotes.

Using the mouse S-49 T-lymphoma cell line in continuous culture, we have isolated and characterized several independently derived clones of mutant cells with altered intracellular dNTP pools. Two such mutants, dGuo-L and dGuo 200-1, have different, defined, allosteric alterations in the M1 subunit of ribonucleoside reductase. A third type of mutant, araC-6-1, is deficient in dCMP deaminase and shows a secondary increase in dCTP concentration with a depletion of its TTP pool. Because each of these three types of mutants possesses deranged dNTP pools due to a distinctly different biochemical defect, one can assess the effect of these pool alterations per se on general mutability. We determined the rates of mutation to 6-thioguanine resistance and dexamethasone resistance for each mutant cell type and two control cell types and found that these mutant cell lines possess increased rates of spontaneous mutation compared with the control cells. This increased mutation rate is characteristic for each mutant and independent of the two genetic markers examined. Furthermore, pharmacologic manipulation demonstrated that the mutation rate is influenced directly by the dNTP pool derangement.

MATERIALS AND METHODS

Materials. 6-Thioguanine, dCTP, dATP, and dGTP were obtained from Sigma; TTP was procured from P-L Biochemicals and [U-14C]cytidine was from Amersham. Agarose was obtained

Abbreviations: dNTP, deoxyribonucleoside triphosphate. HPLC, high-performance liquid chromatography.

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from SeaKem (Rockland, ME), and iodonitrotetrazolium violet was from Calbiochem.

Cell Culture and Maintenance. All cell lines described in this paper are S-49 mouse lymphosarcoma cells (9) which grow in continuous culture in Dulbecco's modified Eagle's medium supplemented with 10% horse serum as described (10, 11).

Selective Cloning. The rates and frequencies of mutation in the five cell lines examined were determined by measuring, within a given population, the number of cells resistant to either 6-thioguanine or dexamethasone. In each experiment the cells were suspended in semisolid (0.30% agarose) culture medium and plated onto 100-mm-diameter plastic dishes containing a monolayer of mouse embryonic fibroblasts (10, 11). To determine the frequency of cells resistant to 6-thioguanine or dexamethasone, cells were plated in 5 ml of medium containing either 60 μM 6-thioguanine or 1 μM dexamethasone at densities of 0.8–1 × 10⁶ cells or 0.8–4 × 10⁶ cells per plate, respectively. Cloning efficiencies were determined by plating small numbers of cells (roughly 200) in nonselective medium. The plates were incubated at 37°C in a humidified CO₂ incubator for 14 days and then covered with a 0.2% solution of the vital stain iodonitrotetrazolium (12). Only those colonies with more than 50 cells and deemed viable (by their ability to reduce the tetrazolium to a formazan) were counted. In plates in which a large number of colonies precluded a complete and accurate manual count, the dish was placed on a grid of 1-cm² squares. The viable clones within seven randomly selected squares were counted and this number was corrected to the actual surface area of the dish.

Determination of Intracellular dNTP Concentrations. The dNTP concentrations in HClO₄ extracts of each cell line were determined by high-performance liquid chromatography (HPLC) (13). Cells in the exponential phase of growth were harvested, washed with chilled buffered saline, and extracted in 0.6 M HClO₄, the supernatant was immediately neutralized with KOH. After periodation (14, 15), the extracts were injected into an HPLC system composed of an Altex Partisil SAX ion exchange column and eluted isocratically with 0.40 M ammonium phosphate, pH 3.4/1.5% acetonitrile at a flow rate of 2 ml/min. Comparison of absorbances at 280 and 254 with those of dNTP standard solutions allowed identification of the dNTPs and determination of their concentrations in each periodated extract.

In Vivo Conversion of Cytidine to Thymidine Nucleotides. The ability of S-49 cells to convert deoxycytidylate to deoxyuridine- and thymidine-containing nucleotides was assayed by the procedure of Maybaum (16). Briefly, this method determines the flux of exogenous precursor [U-¹⁴C]cytidine (485 μCi/μmol) into cytosine-, uracil-, and thymine-containing nucleotides in intact cells and quantitates the CDP reductase and dCMP deaminase activities. The disposition of radiolabeled cytidine was measured in triplicate in 100-ml cultures of exponentially growing NSU-1 and araC-6-1 cells at a density of 10⁶ cells per ml.

RESULTS

Molecular Defects. Previous studies in this laboratory focused attention on the cytotoxicity induced by altered dNTP pools in mouse T-Lymphoma (S-49) cells in continuous culture (17, 18). These studies have been aided by the selection, isolation, and characterization of S-49 cells with specific mutations (14, 19–21). From the same purine nucleoside phosphorylase-deficient parent (NSU-1), two mutants, dGuo-L and dGuo-200-1, were selected independently for resistance to the cytotoxic effect of deoxynanosine. Their biochemical phenotypes, defined in detail previously (14, 21), are a consequence of different alterations in the regulatory properties of their ribonucleotide reductase activities. In each case the specific defect resides in a different nucleotide binding site of the M1 subunit of the reductase molecule (22). The reduction of CDP to DCDP in the dGuo-L cell line has lost normal sensitivity to feedback inhibition by dGTP and TTP (specificity site alteration) (14); that for the dGuo-200-1 cell line is insensitive to dATP (activity site alteration) (21).

The mutant araC-6-1 was selected by virtue of its resistance to arabinosylcytosine and was distinguished from other (largely deoxycytidine kinase-deficient) clones resistant to arabinosylcytosine by its resistance to killing by 50 μM thymidine. araC-6-1 cells contain a ribonucleotide reductase with normal feedback sensitivities to dATP, dGTP, and TTP and normal Km and Kₘ values for CDP and ATP. However, the capacity of these cells to convert precursor [U-¹⁴C]cytidine to dUMP- and thymine-containing nucleotides was no more than 5% of the rate found for the NSU-1 parental cells (0.32 ± 0.07 and 5.56 ± 2.06 nmol of dCMP deaminated per hr per 10⁷ cells, respectively). Thus, the araC-6-1 cells are severely deficient in vivo activity of dCMP deaminase. The biochemical phenotype of araC-6-1 confirms previous observations that dCMP deamination represents a major source of endogenous thymine deoxynucleotides and an important route of efflux from intracellular deoxycytidylate nucleotide pools (23). However, unlike previously described dCMP deaminase deficient mutants (4, 24), araC-6-1 cells are prototrophic for thymidine because they were selected in its absence.

dNTP Pools. The intracellular dNTP pools for each mutant were determined by HPLC. dGuo-L cells have the least altered endogenous dNTP levels; araC-6-1 cells have markedly increased dCTP, reciprocally decreased TTP, and relatively normal purine deoxynucleotide concentrations (Fig. 1). The dGuo-200-1 cells have marked increases in all dNTP pools.

Mutation Frequency. We initially chose dGuo-L cells to study the effects of altered endogenous dNTP pools on spontaneous mutation frequency. In these experiments, small numbers (<10⁶) of dGuo-L and parental NSU-1 cells were grown for several days in hypoxanthine/amethopterin/thymidine me-

![Fig. 1. Intracellular dNTP concentrations in three mutant cell lines relative to that in parental NSU-1 cells. Values for the mutant lines were determined by HPLC in duplicate experiments. The absolute dNTP concentrations (mean ± SD) determined in triplicate for wild type and in quintuplicate for NSU-1 were dATP, 14.8 ± 1.78 and 14.4 ± 3.9 nmol/10⁶ cells; TTP, 14.5 ± 2.5 and 8.7 ± 5.0; dGTP, 28.3 ± 1.1 and 24.0 ± 6.4; and dCDP, 8.4 ± 2.0 and 9.6 ± 3.9. *dATP, □, TTP, ■, dGTP, □, dCDP, *.
dium to eliminate all hypoxanthine phosphoribosyltransferase-deficient cells, allowed to expand further for 1 week in folic acid medium (14, 25, 26), and then plated selectively in 6-thioguanine to measure the frequency of putative hypoxanthine phosphoribosyltransferase-deficient cells in the expanded population. From nine independent experiments, the mean (± SD) frequency of 6-thioguanine-resistant cells, expressed as variants per 10^6 cells, was 0.29 ± 0.27 for NSU-1 and 6.29 ± 2.65 for dGuo-L. Six of the 6-thioguanine-resistant clones were picked and expanded. All were resistant to 6-thioguanine and demonstrated decreased uptake of radiolabeled hypoxanthine. These findings are in agreement with previous experiments demonstrating that virtually all S-49 cell mutants selected in 6-thioguanine are deficient in hypoxanthine phosphoribosyltransferase activity (10).

**Mutation Rates and Fluctuation Analyses.** In order to determine the effects of altered dNTP pools on mutagenesis, we conducted a classic fluctuation analysis (27) and determination of mutation rates (27–29) in five cell lines (wild type, NSU-1, dGuo-L, araC-6-1, and dGuo-200-1) by using two independent genetic markers, 6-thioguanine resistance and dexamethasone resistance (11, 30). For each cell line, six small cultures (<10^3 cells) were expanded in separate flasks and ultimately plated in 6-thioguanine and in dexamethasone. Sibley and Tomkins (30) have characterized the phenotypes of cells resistant to dexamethasone and have shown that the preponderance of the mutants have altered cytoplasmic receptors for dexamethasone. The results (Table 1) demonstrate extreme variation in the number of clones in each plate for a given cell line and selective agent and also show poor correlation between the numbers of 6-thioguanine- and dexamethasone-resistant clones for a given culture. Thus, the data in Table 1 and the mean and variance (not shown) for each cell line and selective agent meet the criteria of Luria and Delbruck (27) for establishing that our results are a measure of random, spontaneous, and independent events (i.e., mutations).

Using the data collected in this experiment, we determined the spontaneous mutation rates in the five cell lines for the two selective agents examined (Table 2) (27, 29). Comparison of these results with Fig. 1 shows a rough correlation between the degree of perturbation in endogenous dNTP pools and the relative increase in spontaneous mutation rates. The latter is reasonably characteristic of the mutant line and independent of the two markers examined.

**Manipulation of Endogenous dNTP Pools.** The NSU-1 and araC-6-1 cells have the capacity to accumulate exogenously supplied thymidine in the form of TTP, to which their CDP-reducing activities are normally sensitive. Thus, exogenous thymidine increases intracellular TTP levels in these cell lines and depletes their dCTP pools, thereby normalizing the dNTP profile of araC-6-1 cells and perturbing the levels in NSU-1 cells. This allows one to determine the effects of intracellular dNTP pool alteration on mutability. Small cultures of the NSU-1 and araC-6-1 cells were expanded continuously either in medium containing 50 μM thymidine and 20 μM deoxycytidine (the latter prevents thymidine cytotoxicity) or in medium with no deoxynucleoside added (control). The cultures were then cloned selectively to determine the rates of mutation to 6-thioguanine resistance as described above for the determination of spontaneous mutation rate. At the time of plating, portions of each culture were collected and their intracellular dNTP concentrations were measured by HPLC. Exogenous thymidine ameliorated the derangement of endogenous dNTP pools in araC-6-1 cells and diminished the mutation rate (Table 3). Conversely, the identical treatment of NSU-1 cells perturbed dNTP pools and enhanced the rate of mutation to 6-thioguanine resistance.

**DISCUSSION**

We have described increased spontaneous mutation rates in three mammalian mutant cells with deranged endogenous dNTP pools, in each case due to a different biochemical defect. These cell lines have distinct advantages for studying the effects of altered dNTP concentrations on DNA replication. They provide a means for the analysis of in situ mutation in a homologous mammalian system, as yet not available in the reconstituted models of DNA synthesis. The derangement of dNTP pools is persistent and well defined for each mutant cell line, obviating

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**Table 1. Resistance to 6-thioguanine and dexamethasone**

<table>
<thead>
<tr>
<th>Cell</th>
<th>Agent</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>( \frac{\Sigma r_{raw}}{N} )</th>
<th>( \sum r_{raw} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>SGua</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Dex</td>
<td>9</td>
<td>24</td>
<td>40</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>82</td>
<td>92</td>
</tr>
<tr>
<td>NSU-1</td>
<td>SGua</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Dex</td>
<td>11</td>
<td>26</td>
<td>13</td>
<td>60</td>
<td>1</td>
<td>5</td>
<td>116</td>
<td></td>
</tr>
<tr>
<td>dGuo-L</td>
<td>SGua</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>19</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Dex</td>
<td>51</td>
<td>253</td>
<td>79</td>
<td>98</td>
<td>5</td>
<td>14</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>araC-6-1</td>
<td>SGua</td>
<td>—</td>
<td>8</td>
<td>6</td>
<td>3</td>
<td>34</td>
<td>18</td>
<td>69</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Dex</td>
<td>1140</td>
<td>1150</td>
<td>410</td>
<td>55</td>
<td>780</td>
<td>113</td>
<td>3,468</td>
<td></td>
</tr>
<tr>
<td>dGuo-200-1</td>
<td>SGua</td>
<td>305</td>
<td>41</td>
<td>33</td>
<td>81</td>
<td>11</td>
<td>—</td>
<td>471</td>
<td>471</td>
</tr>
<tr>
<td></td>
<td>Dex</td>
<td>860</td>
<td>3136</td>
<td>4661</td>
<td>4512</td>
<td>3040</td>
<td>3637</td>
<td>19,846</td>
<td></td>
</tr>
</tbody>
</table>

The frequency of cells resistant to 6-thioguanine (SGua) and dexamethasone (Dex) was determined for each culture by counting the number of viable clones on the corresponding plates 14 days after selective cloning in semisolid medium. Cells were originally plated at densities of 10^4 and 4 x 10^6 cells per plate for 6-thioguanine and dexamethasone, respectively.

**Table 2. Determination of spontaneous mutation rates**

<table>
<thead>
<tr>
<th>Cell</th>
<th>Agent*</th>
<th>( \Sigma r_{raw} )</th>
<th>( N )</th>
<th>( \Sigma r_{calc} )</th>
<th>Mutation rate ( a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>SGua</td>
<td>6</td>
<td>1.93 x 10^8</td>
<td>26</td>
<td>0.56 x 10^-7</td>
</tr>
<tr>
<td></td>
<td>Dex</td>
<td>89</td>
<td>1.93 x 10^8</td>
<td>890</td>
<td>0.90 x 10^-6</td>
</tr>
<tr>
<td>NSU-1</td>
<td>SGua</td>
<td>0</td>
<td>1.86 x 10^8</td>
<td>&lt;4</td>
<td>&lt;0.18 x 10^-7</td>
</tr>
<tr>
<td></td>
<td>Dex</td>
<td>116</td>
<td>1.86 x 10^8</td>
<td>1,214</td>
<td>1.21 x 10^-6</td>
</tr>
<tr>
<td>dGuo-L</td>
<td>SGua</td>
<td>19</td>
<td>1.89 x 10^8</td>
<td>81</td>
<td>1.33 x 10^-7</td>
</tr>
<tr>
<td></td>
<td>Dex</td>
<td>500</td>
<td>1.89 x 10^8</td>
<td>5,320</td>
<td>4.21 x 10^-6</td>
</tr>
<tr>
<td>araC-6-1</td>
<td>SGua</td>
<td>69</td>
<td>1.23 x 10^8</td>
<td>228</td>
<td>4.59 x 10^-7</td>
</tr>
<tr>
<td></td>
<td>Dex</td>
<td>3,648</td>
<td>1.45 x 10^8</td>
<td>29,783</td>
<td>25.1 x 10^-6</td>
</tr>
<tr>
<td>dGuo-200-1</td>
<td>SGua</td>
<td>471</td>
<td>1.37 x 10^8</td>
<td>1,743</td>
<td>22.2 x 10^-7</td>
</tr>
<tr>
<td></td>
<td>Dex</td>
<td>19,846</td>
<td>1.61 x 10^8</td>
<td>179,909</td>
<td>114 x 10^-6</td>
</tr>
</tbody>
</table>

\( \Sigma r_{calc} \) is the sum of clones counted in all plates for a given cell line and selective agent (Table 1). \( N \) is the total number of cells in cultures 1–6 (Table 1) for a given cell line at the time of plating. Correction for the fraction of \( N \) plated (0.31–0.41 for 6-thioguanine and 0.13–0.19 for dexamethasone) and the cloning efficiency (0.74) yields:

\[
\Sigma r_{calc} = (\Sigma r_{raw} \times N)/\text{total cells plated} \times \text{cloning efficiency}.
\]

\( \Sigma r_{calc} \) is related to the mutation rate, \( a \) (mutations per cell per generation), by the equation derived by Delbruck and tested by Luria (27):

\[
\Sigma r_{calc} = aN\ln(1 + aN).
\]

The solutions for \( a \) were derived by an iterative process.
Table 3. Manipulation of dNTP pools and mutation rates

<table>
<thead>
<tr>
<th>Cell</th>
<th>dCTP/TTP</th>
<th>Mutation rate α</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSU-1</td>
<td>Control</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>+ dCyd/dThd</td>
<td>0.31</td>
</tr>
<tr>
<td>araC-6-1-1</td>
<td>Control</td>
<td>41.50</td>
</tr>
<tr>
<td></td>
<td>+ dCyd/dThd</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Small cultures of NSU-1 and araC-6-1 cells were expanded for 10 days in either control medium or medium supplemented with 50 µM thymidine (dThd) and 20 µM deoxycytidine (dCyd). A portion of each culture was selectively cloned to determine the rate of mutation, α mutations per cell per generation, to 6-thioguanine resistance; another portion was used to determine the intracellular dNTP concentrations at the time of plating. The values for dATP and dGTP were changed <10% by the presence of dCyd and dThd.

the use of exogenous agents (e.g., bromodeoxyuridine or thymidine) which have variable effects and which increase the chance that undefined exogenous or metabolically generated products might influence the observed mutation rates.

Two of the mutant lines studied are heterozygous for mutation in one of the alleles coding for the ribonucleotide reductase M1 subunit (22, 31), and their dNTP derangements are a consequence of the mutational alterations in feedback sensitivity of the reductase. It is possible that this mutation may confer a mutator phenotype directly through some specific function of ribonucleotide reductase other than the production of deoxyribonucleotides [e.g., participation in the replication complex (32)]. However, the araC-6-1 mutant demonstrates that dNTP pool derangement alone is sufficient to increase the spontaneous mutation rate. Furthermore, exogenous deoxyribonucleotides which normalize the dNTP pool derangement in araC-6-1 cells suppress their mutator phenotypes; identical treatment of the parental cells perturbs their dNTP pool and increases their rate of spontaneous mutation. Clearly, whether the consequence of any of the three genetic alterations or of pharmacologic manipulation, abnormal dNTP pools are associated with mutator phenotypes. This correlation seems to establish, in eukaryotes, a cause-and-effect relationship; this relationship has been defined in cell-free prokaryotic systems (2, 33, 34).

The araC-6-1 mutant has a biochemical and mutator phenotype closely resembling that of all three cell lines studied by Meuth et al. (5), for which reductase mutations were postulated but not demonstrated. We suggest that those thy “CHO cells (4, 5) have increased dCTP pools and thymidine auxotrophy due to a decreased ability to deaminate dCMP, precisely as described for other cell lines by de Saint Vincent et al. (24).

Recent in vitro studies using the purified T4 replication proteins or E. coli DNA polymerases to copy amber mutant dX174 templates have shown high synthetic fidelity in the context of “balanced” dNTP concentrations and a marked influence of precursor pool bias on the induction of specific transitions or transversions (2, 3, 33, 34); our in vivo results and those of Meuth et al. (5) are in accord with these findings. Several determinants of eukaryotic replicative fidelity [e.g., base addition, proofreading (if it exists), or nonscheduled (repair) synthesis] could be affected by alterations in the endogenous dNTP pools. One simple explanation for this effect is competition between the correct and incorrect deoxyribonucleotides at sites of base addition (33, 34). However, dCTP is thought to be a positive effector of DNA synthesis (31), implying that the increased dCTP pools common to these mutant cell lines may accelerate DNA polymerization and thereby diminish the efficacy of an as yet to be defined editing mechanism (29). The mutagenic influence of exogenous thymidine on parental cell lines lends support to the first hypothesis, whereas the antimutator effect of this treatment on the araC-6-1 cells does not discriminate between the proposed mechanisms. The greater increase in spontaneous mutation rates of the dGua-200-1 cells is compatible with either the base competition or the accelerated polymerization hypothesis because these cells have imbalances of both purine and pyrimidine deoxyribonucleotides (unlike araC-6-1) as well as an increased dCTP pool. Knowing the specific types of mutations preferentially generated by each of these mutant cell lines should lend insight into the mechanisms that maintain the high fidelity of eukaryotic DNA replication.

Thus, mutations affecting genetic functions distinct from the immediate DNA synthetic apparatus can generate mutator phenotypes in mammalian cells. Similar mutant alleles may exist in humans and, by diminishing DNA replicative fidelity, could be responsible for the enhanced rates of carcinogenesis affiliated with specific genetic diseases and found in various pedigrees (35).

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