Effects of Antileukemia Agents on Nuclear Matrix-bound DNA Replication in CCRF-CEM Leukemia Cells


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ABSTRACT

The effects of various antileukemic agents on DNA replication associated with the nuclear matrix were investigated in CCRF-CEM leukemia cells. Residual nuclear matrices were prepared by sequential treatment of nuclei with 0.5 M NaCl; DNase I, and Triton X-100 and contained 1-5%, 10, and 37% of the total nuclear DNA, protein, and phospholipid, respectively. In control cells pulse-labeled for 45 s with ³H]thymidine, the specific activity of nascent DNA was four-fold greater in the nuclear matrix fraction relative to the specific activity of the high salt-soluble (nonmatrix) DNA fraction. Pulse-labeling and reconstitution experiments indicated that this enrichment of newly replicated DNA on the nuclear matrix did not result from aggregation of nascent DNA with the matrix. A 2-h incubation of tumor cells with either 0.1 μM teniposide (VM-26), 0.2 μM VM-26, or 0.5 μM amssacrine (m-AMSA) reduced the relative specific activity of nascent DNA on the nuclear matrix by 59, 61, and 54%, respectively, compared to control cells. In contrast hydroxyurea and cytosine arabinoside, at concentrations that markedly inhibited total nuclear DNA synthesis, did not decrease the relative specific activity of newly replicated DNA on the matrix. The results provide evidence that the antiproliferative effects of the DNA topoisomerase II inhibitors, VM-26 and m-AMSA, are localized on the nuclear matrix of CCRF-CEM leukemia cells.

INTRODUCTION

Studies on the modes of action of anticancer agents have revealed that many of the clinically important agents inhibit tumor cell growth by interfering with DNA replication. However, the actual mechanisms by which anticancer agents inhibit the synthesis of DNA have often been difficult to define, partly because of the incomplete knowledge of the complex processes involved in eukaryotic DNA replication. Recent evidence indicated that the nuclear matrix of eukaryotic cells has an important role in chromatin organization and various nuclear functions including DNA replication (1-8). Nuclear matrix proteins have been shown to bind tightly to DNA and to topologically constrain the interphase chromatin as supercoiled loops, thereby organizing the DNA into discrete replicating units (4-6, 9, 10). In addition, the nuclear matrix also contains specific binding sites for some of the enzymes involved in DNA replication including DNA polymerase α (11-15), DNA primase (16, 17), and DNA topoisomerase II (5, 18). The possibility that these matrix-bound enzymes function within the nucleus as DNA replication complexes was supported by the observations that nuclear matrices from various types of cells were isolated with 1.5 M NaCl, DNase I, and Triton X-100 and contained 1-5%, 10, and 37% of the total nuclear DNA, protein, and phospholipid, respectively. In control cells pulse-labeled for 45 s with ³H]thymidine, the specific activity of nascent DNA was four-fold greater in the nuclear matrix fraction relative to the specific activity of the high salt-soluble (nonmatrix) DNA fraction. Pulse-labeling and reconstitution experiments indicated that this enrichment of newly replicated DNA on the nuclear matrix did not result from aggregation of nascent DNA with the matrix. A 2-h incubation of tumor cells with either 0.1 μM teniposide (VM-26), 0.2 μM VM-26, or 0.5 μM amssacrine (m-AMSA) reduced the relative specific activity of nascent DNA on the nuclear matrix by 59, 61, and 54%, respectively, compared to control cells. In contrast hydroxyurea and cytosine arabinoside, at concentrations that markedly inhibited total nuclear DNA synthesis, did not decrease the relative specific activity of newly replicated DNA on the matrix. The results provide evidence that the antiproliferative effects of the DNA topoisomerase II inhibitors, VM-26 and m-AMSA, are localized on the nuclear matrix of CCRF-CEM leukemia cells.

MATERIALS AND METHODS

Materials. The human CCRF-CEM leukemic cell line was provided by Dr. Joseph R. Bertino of the Yale University School of Medicine, and was propagated at 37°C under 95% air-5% CO₂ in Fischer’s medium supplemented with 10% heat-inactivated horse serum, penicillin (20,000 units/liter), and streptomycin (20 mg/liter). SeaKem GTG agarose was purchased from FMC Corp., Rockland, ME. Tissue culture medium, serum, and antibiotics were obtained from Grand Island Biological Company, Grand Island, NY. Cells were checked periodically for Mycoplasma contamination with the Gen-Probe Mycoplasma ribosomal RNA hybridization kit obtained from Fisher Scientific Co., Raleigh, NC. Dithiothreitol, diphenylamine, ethidium bromide, hydroxyurea, and all other deoxyribonucleosides were obtained from Sigma Chemical Co., St. Louis, MO. VM-26 was a gift of Dr. William T. Bradner of Bristol Myers Co., Syracuse, NY, and Ara-C was provided by Dr. J. Courtland White of Bowman Gray School of Medicine. m-AMSA was obtained from the National Cancer Institute. Distilled nucleic acid-grade phenol and a 1-kilobase DNA ladder were obtained from Bethesda Research Laboratories, Gaithersburg, MD. Coomassie brilliant blue G-250 was purchased from Eastman Kodak Co., Rochester, NY. [Methyl-³H]TdTd and [2-¹⁴C]dTdt with specific radioactivities of 7-20 and 0.056 Ci/mmol, respectively, were purchased from Moravek Biochemicals, Brea, CA. RNase-free pancreatic DNase I with a specific activity of 2,200 Kunitz units per mg of protein was purchased from Cooper Biomedical, Freehold, NJ. Low salt buffer consisted of 10 mM Tris-HCl (pH 7), 1 mM MgCl₂, 10 mM NaCl, and 1 mM PMSF. High salt buffer (1.5 M) consisted of 10 mM Tris-HCl (pH 7), 0.6 mM MgCl₂, 1.5 mM NaCl, and 1 mM PMSF. High salt buffer (3 M) consisted of 10 mM Tris-HCl (pH 7), 0.2 mM MgCl₂, 3 mM NaCl, and 1 mM PMSF.

Isolation of the Nuclear Matrix. Logarithmically growing CCRF-CEM cells were precultured for 72 h (approximately three doubling times) with 30 mM [¹⁴C]dTdt (final specific radioactivity of 0.056 Ci/mmoll) to uniformly radiolabel the DNA. The tumor cells were then harvested by centrifugation at 37°C, and the pellets washed at 37°C with 0.5 M Tris-HCl (pH 7), 1 mM MgCl₂, 10 mM NaCl, and 1 mM PMSF. High salt buffer (1.5 M) consisted of 10 mM Tris-HCl (pH 7), 0.6 mM MgCl₂, 1.5 mM NaCl, and 1 mM PMSF.

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1 The abbreviations used are: VM-26, 4'-demethylepipodophyllotoxinmethylidenedene-d-glucoside (teniposide); m-AMSA, 4'-(9-acridinylamino)-1-methanesulfofon-m-anisidide; Ara-C, 1-β-D-arabinofuranosylcytosine (cytosine arabinoside); dTdT, 2'-deoxythymidine; PMSF, phenylmethylsulfonyl fluoride; TCA, trichloroacetic acid; SDS, sodium dodecyl sulfate.

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on ice for 15 min, and then disrupted with 25 strokes in a Dounce homogenizer. The crude nuclear preparation was layered over 10 ml of 45% sucrose (w/v), and then centrifuged at 1,900 xg for 30 min at 2°C. The purification was monitored by phase contrast microscopy following Giemsa staining. Two hundred structures were counted of which 95% were intact nuclei with well defined borders free of visible cytoplasmic contamination, 4% were whole cells, and 1% were fractured nuclei.

The procedure followed for the isolation of the nuclear matrix was a modification of that described by Pardoll et al. (2). Purified nuclei were gently resuspended in 1 ml of low salt buffer at 4°C. One ml of 3 M high salt buffer was added over a period of 1 h with gentle mixing to yield a final concentration of 1.5 M NaCl. The samples were then incubated for an additional 30 min on ice, and then placed in a water bath at 37°C. The DNA was digested by the addition of 250 units of RNase-free pancreatic DNase I. Preliminary experiments indicated that between 95 and 99% of the total 14C-labeled nuclear DNA was made aqueous soluble under these conditions, although greater than 97% of this DNA could still be precipitated with ice-cold 10% TCA. The samples were then centrifuged and the supernatants were removed for counting of the 3H and 14C labels. Stopping and washing the pellets once with 1 ml of 1.5 M high salt buffer, once with 1 ml of low salt buffer containing 1% (v/v) Triton X-100, and then immediately resuspended and washed with 1 ml of low salt buffer. The matrix and nonmatrix fractions were precipitated in ice-cold 10% TCA (w/v) for 1 h, centrifuged, and resuspended in 500 μl of 0.5 N KOH for 1 h at 37°C to hydrolyze the RNA. All centrifugations were at 4°C with 1 ml of low salt buffer containing 1% (v/v) Triton X-100, and then disrupted with 25 strokes in a Dounce homogenizer with 3 M KOH for 1 h at 37°C to hydrolyze the DNA. All centrifugations were at 13,000 x g for 30 min at 4°C. The samples were then centrifuged and the supernatants (nonmatrix fractions) were removed for counting of the 3H and 14C labels. Spillover of 14C radioactivity into the 3H channel or vice versa was observed following calibration of the liquid scintillation counter for dual label counting. In some experiments the DNA concentrations in the nuclear matrix and nonmatrix fractions were estimated by the diphenylamine colorimetric assay as described by Giles and Myers (23) using 2'-deoxyadenosine as standard. In these experiments the prelabeling of DNA for 72 h with [3H]dThd was omitted. Protein concentrations were estimated by the Coomassie blue staining method (24) and lipid phosphorus was quantitated as previously described (25).

Purification and Analysis of Nuclear Matrix-bound DNA. Nuclear matrices from 6 x 10^6 cells were incubated for 2 h at 60°C in 10 mM Tris-HCl (pH 8.0) buffer containing 0.2% sodium dodecyl sulfate (w/v), 20 mM EDTA, and 100 μg/ml proteinase K. At the end of the incubation, the nuclei were washed with 1 ml of 1 M NaCl and 100 mM Tris-HCl (pH 8), 2 mM EDTA, and 0.5 μg/ml ethidium bromide as the running buffer. Double-stranded DNA restriction fragments from 75 to 12,000 basepairs were used as molecular weight markers.

Mixing Experiments. Logarithmically growing tumor cells (1.2 x 10^6) were pulse-labeled for 72 h with [3H]dThd and then pulse-labeled for 45 s with [3H]dThd. Purified nuclei were prepared as described above and triplicate aliquots, each containing 2 x 10^6 nuclei, were resuspended in 500 μl of low salt buffer. One of the aliquots was mildly sonicated with three 15-s bursts at 15 W using a Branson 200 sonifier equipped with a microtip. This sample was precipitated overnight at -20°C in ethanol containing 150 mM NaCl, and the chromatin fragments were isolated following centrifugation at 13,000 x g for 30 min at 4°C. Nuclear matrix DNA and nonmatrix DNA were isolated and purified from the remaining aliquots as described above. In the mixing experiments the radiolabeled chromatin fragments, nonmatrix DNA, and nuclear matrix DNA were added in triplicate to 2 x 10^7 unlabeled nuclei. Nuclear matrices were prepared from the unlabeled nuclei, and the specific activity of the nascent DNA on the nuclear matrix ([H]/[C]) was calculated to determine any nonspecific binding of DNA to the matrix.

Electron Microscopy. For transmission electron microscopy nuclei and nuclear matrices were fixed in 3% glutaraldehyde, postfixed in 1% osmium tetroxide, and stained with lead citrate and uranyl acetate as previously described (27). Sections of 60-mm thickness were examined in a Philips EM-400 electron microscope. Nuclei and nuclear matrices were prepared for scanning electron microscopy by fixation of the samples on glass coverslips followed by coating of the glass surfaces in a D. C. sputterer with gold-palladium (28).

RESULTS

Characterization of the Nuclear Matrix. Initial studies involved a morphological and biochemical characterization of the nuclear matrix, since similar studies with CCRF-CEM leukemia cells had not been previously reported. In addition, it was first necessary to demonstrate that newly synthesized DNA was specifically bound and enriched on the nuclear matrix before the effects of anticancer agents on nuclear matrix-bound DNA synthesis could be investigated. Scanning electron microscopy of whole nuclei revealed nearly spherical structures of about 7 μm in diameter (Fig. 1A). Nuclear matrices that were prepared from CCRF-CEM cells by high salt extraction and DNase I digestion of purified nuclei contained 1-5, 10, and 37% of the total nuclear DNA, protein, and phospholipid, respectively. The removal of most of the nuclear protein, phospholipid, and DNA permitted the visualization of the residual nuclear framework or matrix. The nuclear matrices were of similar size and shape as unfractionated nuclei but had a sponge-like appearance due to the presence of numerous pores of varying sizes (Fig. 1B). Comparison of whole nuclei (Fig. 1C) and nuclear matrices (Fig. 1D) by transmission electron microscopy indicated that the matrix lacked much of the chromatin of the whole nucleus and contained only small remnants of the nucleolus and nuclear envelope. The matrix consisted mainly of a residual framework of fibrils and granules that was connected to a single peripheral lamina. The proteins solubilized from the nuclei and nuclear matrices were separated by SDS polyacrylamide gel electrophoresis (Fig. 2). Compared to whole nuclei, the nuclear matrices of CCRF-CEM cells were extensively depleted of histones (M, 13,000–20,000) but were relatively enriched in M, 60,000–70,000 proteins. These results were similar to those previously obtained with rat liver and other cells (1, 3, 7).

It was reasoned that if DNA replication occurred at specific sites on the residual matrix framework, then the insoluble nuclear matrix should be enriched in nascent DNA compared to the high salt-soluble (nonmatrix) DNA fraction. As a means of testing this hypothesis, CCRF-CEM cells were prelabeled to the high salt-soluble (nonmatrix) DNA fraction. As a means of testing this hypothesis, CCRF-CEM cells were prelabeled to the high salt-soluble (nonmatrix) DNA fraction. As a means of testing this hypothesis, CCRF-CEM cells were prelabeled...
measured by the diphenylamine colorimetric assay instead of by \[^{14}C\]dThd prelabeling, a 5.1-fold enrichment of newly synthesized DNA on the nuclear matrix (1018 \(^3\)H dpm/\(\mu\)g of DNA) was observed compared to the nonmatrix DNA (201 \(^3\)H dpm/\(\mu\)g of DNA). Thus, regardless of the method used to determine DNA content, a similar degree of enrichment of newly synthesized DNA on the nuclear matrix was observed. In all subsequent studies, the relative amounts of DNA were quantitated following \[^{14}C\]dThd prelabeling because of the much greater sensitivity of the radiochemical technique compared to the colorimetric assay in detecting the small amounts of DNA bound to the nuclear matrix.

It was important to note that when CCRF-CEM cells were pulse-labeled for 20 min with \(^3\)H)dThd as opposed to 45 s, no enrichment of the \(^3\)H radiolabel was seen on the nuclear matrix compared to the nonmatrix fraction (Table 1). During the 20-min continuous labeling period a large fraction of the \(^3\)H)dThd would have become incorporated into high molecular weight DNA that was no longer replicating. Therefore, the results presented in Table 1 were consistent with the proposed concept (2, 9, 29) that DNA replication takes place at sites on the nuclear matrix, and that previously replicated DNA moves progressively away from these sites into the nonmatrix (high salt-soluble) DNA according to the rate of replication fork movement.

Control Experiments. The results from several types of control experiments indicated that the enrichment of nascent DNA observed on the nuclear matrix was not the consequence of aggregation of the nascent DNA fragments with the nuclear matrix. The repeated isolation of nuclear matrices with a constant relative specific activity of nascent DNA (Tables 1 and 3) suggested that nonspecific association of DNA with the matrix was unlikely. In addition, the \(^3\)H radioactivity in the nuclear matrix fractions shown in Tables 1 and 3 were measured after successive washings of the isolated matrices with high salt buffer containing 1.5 M NaCl, low salt buffer containing 1% Triton X-100, low salt buffer, and 10% TCA.

Reconstitution experiments indicated that nascent DNA did not preferentially bind to the nuclear matrix during the matrix isolation procedure. CCRF-CEM cells were prelabeled with \[^{14}C\]dThd for 72 h and then pulse-labeled for 45 s with \(^3\)H)dThd. The double-labeled chromatin fragments, purified matrix DNA, and nonmatrix DNA were prepared and added to suspensions of unlabeled nuclei. Nuclear matrices were then iso-

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Fig. 1. Electron micrographs of whole nuclei and nuclear matrices from CCRF-CEM leukemia cells. Nuclear matrices were prepared as described in the text and contained 2, 10, and 37% of the total nuclear DNA, protein, and phospholipid, respectively. A and B, scanning electron micrographs of a nucleus and nuclear matrix, respectively; C and D, transmission electron micrographs of a nucleus and nuclear matrix, respectively. Arrow in D, a small remnant of the nuclear envelope. Bars, 1 \(\mu\)m.
that dThd was actually incorporated into DNA at replication sites on the nuclear matrix.

Effects of Antileukemic Agents on Nuclear Matrix-bound DNA Replication. It was of interest to examine the sensitivity of nuclear matrix-bound DNA synthesis to various antileukemic agents. Table 3 shows that brief exposure of CCRF-CEM cells to relatively low concentrations of either VM-26 or m-AMSA resulted in preferential inhibition of $[^{3}H]d$Thd incorporation into nuclear matrix DNA compared to nonmatrix DNA. Newly synthesized DNA in control cells was enriched 4.1-fold in the nuclear matrix fraction compared to the nonmatrix fraction. Following a 2-h incubation of cells with either 0.1 $\mu$M VM-26, 0.2 $\mu$M VM-26, or 0.5 $\mu$M m-AMSA, the relative specific activities of nascent DNA on the nuclear matrix were decreased 59% (1.7-fold enrichment), 61% (1.6-fold enrichment), and 54% (1.9-fold enrichment), respectively. Similar results were obtained when the duration of the $[^{3}H]d$Thd pulse-label was changed from 45 s to either 15, 30, or 90 s (data not shown).

It was important to note that these effects of VM-26 and m-AMSA could not be readily attributable to differences between control and drug treated cells in either dThd transport, dThd phosphorylation, or deoxyribonucleoside triphosphate pool sizes. Drug-induced alterations in these parameters would not be expected to affect the rate of $[^{3}H]d$Thd incorporated into the matrix DNA fraction compared to that incorporated into the nonmatrix DNA fraction within the individual cell. In contrast to the specific effects observed with the DNA topoisomerase II inhibitors, VM-26 and m-AMSA, hydroxyurea and Ara-C did not reduce the relative specific activity of nascent DNA on the matrix. A 2-h exposure of CCRF-CEM cells to 200 $\mu$M hydroxyurea decreased the incorporation of $[^{3}H]d$Thd into nuclear matrix DNA 77% compared to control cells (Table 4). However, $[^{3}H]d$Thd incorporation into the nonmatrix DNA fraction was also decreased 77%, so that no change in the relative specific activity was observed. Likewise, a 2-h incubation of leukemia cells with 0.1 $\mu$M Ara-C produced similar declines in both the matrix and nonmatrix specific activities compared to control cells (Table 4). Hydroxyurea at 400 $\mu$M and Ara-C at 0.03 $\mu$M for 2 h also did not change the ratio of the matrix DNA specific activity to the nonmatrix DNA specific activity (data not shown). These observations indicated that the DNA topoisomerase II inhibitors, in contrast to hydroxyurea and Ara-C, preferentially interfered with DNA replication at sites on the nuclear matrix.

**DISCUSSION**

The identification and partial characterization of the nuclear matrix from various types of eukaryotic cells has provided new insights into the cellular organization and replication of DNA.

![Image](Fig. 2. SDS polyacrylamide electrophoresis gel of proteins present in whole nuclei and nuclear matrices of CCRF-CEM cells. Nuclei and nuclear matrices were isolated as described in "Materials and Methods." The proteins were electrophoresed on a 12% SDS polyacrylamide gel and stained with Coomassie blue as described by Laemmli (32). Lanes NM (nuclear matrices) and N (whole nuclei) were loaded with 31 $\mu$g and 27 $\mu$g of protein, respectively. The apparent molecular masses ($\times 1000$) of the standard proteins (lane S) are shown at the right.)

**Table 1 Nuclear matrix-bound DNA synthesis**

Logarithmically growing CCRF-CEM cells were prelabeled for 72 h in the presence of $[^{14}C]d$Thd, resuspended in fresh medium, and then labeled for either 45 s or 20 min with $[^{3}H]d$Thd. The nuclei were isolated by centrifugation through 45% (w/v) sucrose and the nuclear matrices prepared as described in "Materials and Methods."

<table>
<thead>
<tr>
<th>Radiolabeling procedure</th>
<th>Nuclear DNA fraction</th>
<th>Total DNA ($^{14}C$ dpm)</th>
<th>Newly synthesized DNA ($^{3}H$ dpm)</th>
<th>Specific activity $^{3}H$ dpm to $^{14}C$ dpm</th>
<th>Relative* b</th>
</tr>
</thead>
<tbody>
<tr>
<td>45-s pulse $[^{3}H]d$Thd</td>
<td>Matrix</td>
<td>782</td>
<td>11,218</td>
<td>14.3</td>
<td>4.0 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Nonmatrix</td>
<td>32,085</td>
<td>114,400</td>
<td>3.6</td>
<td>1</td>
</tr>
<tr>
<td>20-min pulse $[^{3}H]d$Thd</td>
<td>Matrix</td>
<td>621</td>
<td>33,121</td>
<td>53.3</td>
<td>0.8 ± 0.1c</td>
</tr>
<tr>
<td></td>
<td>Nonmatrix</td>
<td>29,227</td>
<td>1,945,381</td>
<td>66.6</td>
<td>1</td>
</tr>
</tbody>
</table>

* Ratio of the specific activity of DNA in the nuclear matrix fraction to the specific activity in the nonmatrix fraction.

b Mean ± SD ($N = 3$).

c $P < 0.01$ compared to the relative specific activity of the matrix DNA obtained after a 45-s pulse label with $[^{3}H]d$Thd (two-tailed t test).
Logarithmically growing CCRF-CEM cells were prelabeled for 72 h with [14C]-dThd and then pulse labeled for 45 s with [3H]dThd. Chromatin fragments, nonmatrix DNA, and purified matrix DNA were isolated from the labeled nuclei and then added to unlabeled nuclei. Nuclear matrices were prepared from the unlabeled nuclei as described in "Materials and Methods." In each sample the recovery of either 3H or 14C radioactivity after nuclear matrix isolation was greater than 93%.

Table 2 Binding of chromatin fragments, purified matrix DNA, and nonmatrix DNA to the nuclear matrix

<table>
<thead>
<tr>
<th>Radiolabeled DNA added</th>
<th>Nuclear fraction</th>
<th>Newly synthesized DNA</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[14C] dpm</td>
<td>[3H] dpm</td>
<td>[3H]/[14C] Relative*</td>
</tr>
<tr>
<td>Chromatin fragments</td>
<td>111</td>
<td>2,805</td>
<td>25</td>
</tr>
<tr>
<td>No drug (control)</td>
<td>2,668</td>
<td>91,124</td>
<td>34</td>
</tr>
<tr>
<td>Nonmatrix</td>
<td>79</td>
<td>92</td>
<td>1</td>
</tr>
<tr>
<td>DNA</td>
<td>4,804</td>
<td>11,575</td>
<td>2</td>
</tr>
<tr>
<td>Purified matrix</td>
<td>89</td>
<td>783</td>
<td>9</td>
</tr>
<tr>
<td>DNA</td>
<td>932</td>
<td>14,692</td>
<td>16</td>
</tr>
</tbody>
</table>

* Ratio of the specific activity of DNA in the nuclear matrix fraction to the specific activity in the nonmatrix fraction.

Table 3 Effects of VM-26 and m-AMSA on nuclear matrix-bound DNA replication

Logarithmically growing CCRF-CEM cells were prelabeled for 72 h with [14C]-dThd, resuspended in fresh medium, and then incubated for 2 h with either no drug (control), VM-26, or m-AMSA. The cells were then pulse labeled for 45 s with [3H]dThd and the nuclear matrices prepared as described in "Materials and Methods."

<table>
<thead>
<tr>
<th>Condition</th>
<th>Total DNA (14C dpm)</th>
<th>Newly synthesized DNA (3H dpm)</th>
<th>Specific activity [3H]/[14C] Relative*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matrix</td>
<td>840</td>
<td>13,689</td>
<td>16.3</td>
</tr>
<tr>
<td>Nonmatrix</td>
<td>34,624</td>
<td>138,401</td>
<td>4.0</td>
</tr>
<tr>
<td>VM-26</td>
<td>32,428</td>
<td>77,649</td>
<td>2.2</td>
</tr>
<tr>
<td>Matrix</td>
<td>769</td>
<td>2,214</td>
<td>3.8</td>
</tr>
<tr>
<td>Nonmatrix</td>
<td>34,454</td>
<td>22,917</td>
<td>0.7</td>
</tr>
<tr>
<td>m-AMSA</td>
<td>454</td>
<td>1,162</td>
<td>2.6</td>
</tr>
<tr>
<td>Matrix</td>
<td>21,547</td>
<td>30,005</td>
<td>1.4</td>
</tr>
</tbody>
</table>

* Mean ± SD (N = 4).

Table 4 Effects of hydroxyurea and Ara-C on nuclear matrix-bound DNA replication

Logarithmically growing CCRF-CEM cells were prelabeled for 72 h with [14C]-dThd, resuspended in fresh medium, and then incubated with either no drug (control), Ara-C, or hydroxyurea for 2 h. The cells were then pulse labeled for 45 s with [3H]dThd, and the nuclear matrices prepared as described in "Materials and Methods."

<table>
<thead>
<tr>
<th>Condition</th>
<th>Total DNA (14C dpm)</th>
<th>Newly synthesized DNA (3H dpm)</th>
<th>Specific activity [3H]/[14C] Relative*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matrix</td>
<td>840</td>
<td>13,689</td>
<td>16.3</td>
</tr>
<tr>
<td>Nonmatrix</td>
<td>34,624</td>
<td>138,401</td>
<td>4.0</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(200 μM)</td>
<td>316</td>
<td>1,175</td>
<td>3.7</td>
</tr>
<tr>
<td>Matrix</td>
<td>29,184</td>
<td>25,072</td>
<td>0.9</td>
</tr>
<tr>
<td>Ara-C</td>
<td>289</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>(0.1 μM)</td>
<td>29,184</td>
<td>25,072</td>
<td>0.9</td>
</tr>
<tr>
<td>Matrix</td>
<td>314</td>
<td>289</td>
<td>4.5</td>
</tr>
<tr>
<td>Nonmatrix</td>
<td>44,313</td>
<td>7,947</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* Ratio of the specific activity of DNA in the nuclear matrix fraction to the specific activity in the nonmatrix fraction.

Although the matrix likely functions as a structural component of the nucleus, numerous studies indicate that it also has an important role in various nuclear processes including DNA replication (1–8). Because of the involvement of the nuclear matrix in DNA replication, it seemed reasonable that certain antiproliferative agents would specifically interfere with the function of DNA replication complexes on the nuclear matrix. However, for the most part these potentially important effects have remained unexplored.

Nuclear matrices were prepared from CCRF-CEM cells by sequential treatment of purified nuclei with low salt buffer, high salt buffer, pancreatic DNase I, and Triton X-100. The morphological and biochemical characteristics of the resultant matrices were generally similar to the type III matrices isolated from regenerating rat liver (4). Nuclear matrix preparations from CCRF-CEM cells were 4-fold enriched in newly synthesized DNA compared to the high salt-soluble nonmatrix DNA. The results of the pulse-labeling and reconstitution experiments indicated that this preferential association of newly replicated DNA with the nuclear matrix did not result from aggregation of nascent DNA with the matrix. Using similar matrix isolation and pulse-labeling techniques, others have observed a 2- to 15-fold enrichment of nascent DNA on the nuclear matrices of F4N Friend cells, HeLa cells, regenerating rat liver, and mouse fibroblasts (2, 13, 29, 30).

The results obtained from this study with CCRF-CEM cells provide further information for evaluating the role of the nuclear matrix in DNA replication. A model of DNA replication has been proposed in which DNA replication complexes and replicating DNA loops are attached to the nuclear matrix (2, 9, 29). In this model newly replicated DNA moves progressively away from the matrix-bound replication sites according to the rate of replication fork movement and then becomes incorporated into the high salt-soluble DNA. If this model were appropriate for DNA replication in CCRF-CEM cells, one would expect that a smaller fraction of the newly replicated DNA would have migrated away from the matrix-bound replication sites during a 45-s pulse label than during a 20-min pulse label. Thus, the enrichment of newly replicated DNA that was observed in the nuclear matrix fraction after a 45-s [3H]dThd pulse label, but not after the 20-min pulse label, was consistent with this model.

In order to further characterize nuclear matrix-bound DNA replication and investigate the effects of various antileukemic agents in this system, CCRF-CEM cells were incubated with either VM-26, m-AMSA, hydroxyurea, or Ara-C. It was of considerable interest that only VM-26 and m-AMSA, agents which interfered with the activity of DNA topoisomerase II, reduced the relative specific activity of nascent DNA on the nuclear matrix. This effect was seen after brief exposure of CCRF-CEM cells to relatively low concentrations of either VM-26 or m-AMSA. Hydroxyurea and Ara-C, at concentrations which produced marked inhibition of total nuclear DNA synthesis, did not preferentially block [3H]dThd incorporation into nuclear matrix-bound DNA compared to nonmatrix DNA. We proposed that the effects of VM-26 and m-AMSA on nuclear matrix-bound DNA replication are related to the localization of DNA topoisomerase II activity on the nuclear matrix of CCRF-CEM cells. This proposal was supported by the recent observations that DNA topoisomerase II was a major protein of the Drosophila nuclear matrix (18), and that VM-26-topoisomerase II complexes were bound primarily to newly replicated DNA of tumor cells (31). Moreover, an important role for DNA topoisomerase II on the nuclear matrix was also suggested by reports that the anchoring of supercoiled DNA loops to the nuclear matrix induced torsional stress in the DNA (4, 9).
It was of interest to consider these different drug effects in light of the model of nuclear matrix-bound DNA replication that was discussed above. According to this model all DNA replication takes place on the nuclear matrix, and the presence of newly replicated DNA in the nonmatrix fraction is the result of migration of this DNA from the matrix-bound replication sites. Therefore, one might expect that other antimetabolite inhibitors of DNA replication would have an indirect effect like that observed with hydroxyurea or Ara-C. By blocking to a similar extent DNA replication and the subsequent migration of newly replicated DNA away from the matrix, hydroxyurea and Ara-C would produce no changes in the relative specific activity of the nascent DNA on the matrix. However, the finding that VM-26 and m-AMSA actually decreased the relative specific activity of matrix DNA suggested that these drugs induced direct perturbations in the nuclear matrix DNA replication system. Gasser and Laemmli (5) have provided evidence that DNA topoisomerase II is enriched at the attachment sites of chromatin loops to the Drosophila nuclear matrix. Accordingly, one might speculate that the interference with DNA topoisomerase II activity on the nuclear matrix by VM-26 and m-AMSA would lead to inhibition of the binding of parental and newly replicated DNA to the matrix. As a result of its decreased matrix attachment some of the newly replicated DNA would be more readily extracted during the matrix isolation procedure, and the relative specific activity of the nascent DNA on the matrix would thereby be lowered. Another interesting possibility is that following the interaction of VM-26 or m-AMSA with DNA topoisomerase II on the nuclear matrix, at least some of the residual DNA synthesis no longer takes place on the matrix. These potential effects of VM-26 and m-AMSA represent novel drug actions and are currently under investigation in this laboratory. In summary, it appears likely that the nuclear matrix is an important cellular site at which certain anticancer agents exert their antiproliferative effects.

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REFERENCES

tion of an RNA priming system in nuclear matrix isolated from regenera
21. Fernandes, D. J., and Cranford, S. K. Dissociation of thymidylate biosynthesis from DNA biosynthesis by 5-fluoro-2′-deoxyuridine and 5,8-dideoaza
Effects of Antileukemia Agents on Nuclear Matrix-bound DNA Replication in CCRF-CEM Leukemia Cells
