In vitro DNA dependent synthesis of globin RNA sequences from erythroleukemic cell chromatin

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ABSTRACT

Murine erythroleukemic cells in culture accumulate cytoplasmic globin mRNA during differentiation induced by dimethyl sulfoxide (DMSO). Chromatin was prepared from DMSO induced erythroleukemic cells that were transcribing globin RNA in order to determine whether in vitro synthesis of globin RNA sequences was possible from chromatin. RNA was synthesized in vitro using 5-mercuriuridine triphosphate and exogenous Escheria coli RNA polymerase. Newly synthesized mercurated RNA was purified from endogenous chromatin associated RNA by affinity chromatography on a sepharose sulfhydryl column, and the globin RNA sequence content of the mercurated RNA was assayed by hybridization to cDNA globin. The synthesis of globin RNA sequences was shown to occur and to be sensitive to actinomycin and rifampicin and insensitive to α-amanitin. In contrast, synthesis of globin RNA sequence synthesis was not detected in significant amounts from chromatin prepared from uninduced erythroleukemic cells, nor from uninduced cell chromatin to which globin RNA was added prior to transcription. Isolated RNA:cDNA globin hybrids were shown to contain mercurated RNA by affinity chromatography. These results indicated that synthesis of globin RNA sequences from chromatin can be performed by E. coli RNA polymerase.

INTRODUCTION

The development of systems for in vitro RNA synthesis from eukaryotic chromatin could contribute to our understanding of RNA transcription and its regulation in vivo. However, difficulty in distinguishing between chromatin associated endogenous RNA and RNA newly synthesized in vitro has hindered studies in this area. Early work which used cDNA globin as a probe for globin RNA synthesis from erythroid cells has been criticized for failing to show that the globin RNA sequences assayed by hybridization were newly synthesized. Subsequent studies using labeled ribonucleotides showed that some of the RNA that hybridized to a [3H]cDNA globin probe was newly synthesized [32P] labeled RNA.

Mercurated nucleotides can be utilized in vitro by RNA polymerases to synthesize HgRNA, which can be separated from non-HgRNA by affinity chroma-
In principle, this should solve the problem of separating newly synthesized RNA from endogenous RNA. Using this technique, in vitro synthesis from chromatin has been reported for immunoglobulin, globin, and ovalbumin RNA sequences\(^9,10,11\). However, numerous complications have been described recently which make it probable that these results may have been due to the presence of endogenous RNA sequences rather than to HgRNA newly synthesized from DNA. E. coli RNA polymerase aberrantly transcribes endogenous RNA, and the resulting double stranded RNA hybrid, which contains one strand of endogenous non-HgRNA, copurifies with HgRNA unless it is denatured prior to affinity chromatography\(^12,13\). Another possible way endogenous RNA could be mistakenly identified in these experiments is through aggregation by HgRNA, leading to non-specific trapping of non-HgRNA in sulfhydryl columns\(^14\).

These recent criticisms have raised serious doubts about the reported synthesis of specific RNA sequences from chromatin and make it necessary to re-evaluate in vitro chromatin directed RNA synthesis. We have analyzed the synthesis of globin RNA sequences from chromatin according to the following criteria: synthesis of RNA must be DNA directed, i.e., sensitive to actinomycin, and the isolated RNA:cDNA hybrids must be shown to contain newly synthesized (mercurated) RNA.

Gently prepared chromatin has been shown to have endogenous 5s RNA transcriptional activity which is asymmetric and initiates and terminates correctly\(^15\). We have prepared chromatin by the same method from DMSO induced erythroleukemic cells and have used it to investigate whether E. coli RNA polymerase can synthesize globin RNA sequences in vitro from chromatin that has been isolated from cells that had been transcribing the globin genes.

**MATERIALS AND METHODS**

**Cell Culture:** Murine erythroleukemic cells of the line T3C12\(^16\) were maintained in Dulbecco's modified Eagle's medium supplemented with 2mM glutamine and 10% fetal calf serum. Erythroid maturation was induced for 81 hours with 1.5% (vol/vol) DMSO\(^17\) and then induced cells were harvested for chromatin preparation. When erythroleukemic cells are induced under these conditions, between 50 and 70% of the cells will contain hemoglobin (as determined by benzidine staining\(^18\)) after 5 days.

**Chromatin preparation:** All operations were done at 4°C, using glassware that had been acid washed, baked and siliconized. All buffers were prepared in advance, except that \(\beta\)-mercaptoethanol was added to solutions on the same day the chromatin was prepared. The following buffers were used: Buffer
A - 0.3M sucrose, 2mM Mg (acetate)$_2$, 2mM CaCl$_2$, 2mM $\beta$-mercaptoethanol, 1% Triton - X - 100, 10 mM Tris, pH 8.0; Buffer B - 2M sucrose, 5mM Mg (acetate)$_2$, 2mM $\beta$-mercaptoethanol, 10mM Tris, pH 8.0; Buffer C - 25% glycerol, 5mM Mg (acetate)$_2$, 2mM $\beta$-mercaptoethanol, 50mM Tris, pH 8.0; Buffer D - 10% glycerol, 2mM $\beta$-mercaptoethanol, 10mM Tris, pH 8.0; Buffer E - 12.5mM EDTA, 62.5mM Na acetate, pH 5.1; Buffer F - 10mM EDTA, 50mM Na acetate, pH 5.1.

The procedure followed was a modification of that described by Marzluff and Huang. DMSO induced cells were washed with Dulbecco's phosphate buffered saline and resuspended (10$^7$ cells/ml) in Buffer A. The cells were homogenized in a dounce homogenizer (10 strokes, tight pestle), which breaks the cells but leaves the nuclei intact. An equal volume of Buffer B was added to the homogenate and this mixture was layered over a pad of Buffer B and centrifuged: when a Beckman rotor SW50.1 was used, the pad was 2 ml and the centrifugation was for 45 min. at 48,000g (20,000 rpm); when a Beckman SW27 rotor was used, the pad was 15 ml and the centrifugation was for 80 min. at 48,000g (17,000 rpm).

The pellet of nuclei then was resuspended (at the equivalent of 5 x 10$^7$/ml) in Buffer C. KCl was added to a concentration of 0.12M, and three minutes later the solution was centrifuged at 121g (1000 rpm, Sorvall rotor SS34) for 3 minutes. The nuclei pellet was resuspended (at the equivalent of 5 x 10$^7$/ml) in Buffer D and centrifuged at 121g for 3 min. The nuclei were broken during this centrifugation. The sticky chromatin pellet was washed twice in Buffer D (same volume as above) by resuspending the pellet with a glass rod and centrifuging at 121g for 3 min. The chromatin then was resuspended in Buffer D (at the equivalent of 10$^8$ nuclei/ml) and solubilized by passage through an 18G needle ten times and through a 22G needle 5 times. Solubilized chromatin was used immediately for RNA synthesis.

Chromatin was assayed for DNA content by the diphenylamine method of Burton. RNA and protein content of chromatin was determined by the procedure of Shaw and Huang. Chromatin prepared by the above method from T3C12 had a ratio of DNA:RNA ranging from 6:1 to 3:1 and a ratio of basic protein:acidic protein:DNA of 1:0.5:1.

Synthesis of RNA: RNA was synthesized in a 1 ml reaction mixture containing solubilized chromatin from 5 x 10$^7$ cells (approx. 500 $\mu$g DNA), 100 units E. coli RNA polymerase (Miles Research Products), 1mM GTP, 1mM CTP, 1mM ATP, 0.2mC $^3$H]-CTP (>20 Ci/mmole, New England Nuclear), 0.67mM UTP, 0.33mM HgUTP (PL Biochemicals), 120mM KCl, 12mM $\beta$-mercaptoethanol, 8mM MgCl$_2$, 10% glycerol, and 40 mM Tris at pH 7.9. The reaction was allowed to proceed for
1 hr. at 37°C. At 1:2 ratio of mercuri-UTP:UTP was used to avoid the inhibition of hybridization seen with RNA synthesized with 100% HgUTP13,21.

Isolation of RNA: For RNA isolation 1 ml 10% SDS, 8 ml of Buffer E and 10 ml phenol (redistilled, equilibrated with Buffer F), all prewarmed to 55°C, were added to 1 ml of reaction mixture. This was shaken periodically for 5 minutes at 55°C. Ten ml of chloroform:isoamyl alcohol (24:1) at 55°C were added and the mixture was shaken periodically for 5 minutes more at 55°C. The mixture was centrifuged and the bottom organic phase and interface removed. The aqueous phase was re-extracted three times more with 10 ml chloroform:isoamyl alcohol at room temperature. The aqueous phase then was made 0.3M NaCl by the addition of 1/5 volume of 1.8M NaCl. 2 volumes of absolute ethanol were added and the mixture placed at -20°C overnight to precipitate RNA. The next day the mixture was centrifuged at 12,000g (10,000 rpm, Sorvall rotor SS34) for 30 min. The pellet was resuspended in 1 ml of 58.8 mM Tris, pH 8.0 and either frozen or applied immediately to sulfhydryl columns. As a control for aggregation in the sulfhydryl column due to the presence of HgRNA14, unlabelled HgtRNA (prepared by Dr. Stuart Orkin) was added to the RNA samples which contained none or little synthesized 3H HgRNA; this included samples from synthesis reactions containing either rifampicin, actinomycin, or 1mM UTP without HgUTP. The sample was not DNase treated because omitting this step does not interfere with purification of the HgRNA22.

Affinity column purification of HgRNA: Sulfhydryl Sepharose-6B was prepared exactly as described by Dale and Ward23. Each batch of sulfhydryl sepharose was tested for its ability to bind samples of HgRNA and for nonspecific absorption of non-HgRNA by passage of radioactive non-HgRNA.

The sulfhydryl sepharose used in this work showed nonspecific binding of non-HgRNA of less than one part in 104 and bound about 70% of the HgRNA added to the column. Columns containing 2 mls of sulfhydryl sepharose were prewashed with 5 column volumes of column buffer (0.1M NaCl, 20mM Tris, pH 7.5) plus 0.1M β-mercaptoethanol, 25 column volumes of column buffer, and 12.5 column volumes of column buffer plus 0.5% SDS.

RNA samples (1 ml in 58.8 mM Tris, pH 8.0) were made 0.5% SDS, boiled for 5 minutes, quick cooled on ice, made 0.1M NaCl, and applied to prewashed columns. (RNA samples were boiled to denature double stranded RNA and eliminate the artefact of endogenous RNA in hybrid with HgRNA copurifying with HgRNA on the sulfhydryl column12,13.) After allowing the RNA samples to incubate with the sulfhydryl column for 1 hour at room temperature, the columns were washed with 25 column volumes of column buffer plus 0.5% SDS, 10 column volumes of
0.5% SDS, 10 mM Tris, pH 7.5 at 55°C (hot wash), and 25 column volumes of column buffer. The HgRNA was eluted by washing the column with 5 column volumes of column buffer plus 0.1M β-mercaptoethanol. Fractions containing HgRNA were identified by \( ^3 \)H counts, pooled and made 0.3M NaCl, 100 μg tRNA was added, and the RNA was precipitated overnight at -20°C by the addition of two volumes of absolute ethanol. Precipitated RNA was resuspended in water, lyopholized and frozen at -70°C prior to molecular hybridization.

Molecular hybridization: Preparation of mouse globin RNA, synthesis and purification of globin cDNA, and molecular hybridization were performed as described by Orkin \(^{24} \). The specific activity of the cDNA was approximately \( 4 \times 10^8 \) cpm \( ^{32} \)P/μg. The cDNA is entirely specific for sense globin RNA \(^{25} \).

RESULTS
Chromatin was prepared from induced erythroleukemic cells and RNA was synthesized in parallel in five separate reactions: 1) conditions as described in Materials and Methods. 2) with 1mM UTP and no HgUTP. 3) plus 10 μg actinomycin. 4) plus 1 μg α-amanitin. 5) plus 1 μg rifampicin. The results of these assays are presented in Table I.

When HgUTP was omitted from the synthetic reaction, the total amount of \( ^3 \)H RNA synthesized was larger than when HgUTP was present. This is consistent with previous reports of inhibition of RNA synthesis in the presence of mercurinucleotide \(^9,22 \). The \( ^3 \)H counts recovered from the mercapton wash of the sulfhydryl column from the reaction without HgUTP represent less than 1 part in \( 10^4 \) of the total counts recovered from the column. The globin RNA sequences detected (2 pg) in these samples synthesized without HgUTP represents the background level of endogenous RNA contamination. This contamination is approximately one part in \( 10^4 \) of the endogenous globin RNA that would be expected to be present in chromatin prepared from \( 5 \times 10^7 \) cells (approximately 15 ng globin RNA, assuming a 3:1 ratio of DNA:RNA in prepared chromatin and that 0.01% of the endogenous nuclear RNA is globin RNA \(^{24} \)). Since HgtRNA was added to this sample prior to column purification, aggregation or trapping of non-HgRNA in the column due to the presence of HgRNA does not appear to occur. However, it is possible that aggregation by bulk synthesized RNA cannot be duplicated by HgtRNA. Therefore, we have attempted to answer this question by another control experiment described below.

Synthesis of total RNA and the synthesis of globin RNA sequences were inhibited in the presence of actinomycin. This indicates that double stranded DNA rather than single stranded RNA is the template for synthesis \(^{13} \).
Table I

Hybridization of cDNA Globin to Purified HgRNA Synthesized from Induced Erythroleukemic Chromatin

<table>
<thead>
<tr>
<th>A RNA synthesis reaction</th>
<th>B cpm (32p) of RNA synthesized (1)</th>
<th>C % of RNA synthesis based on reaction #1 (2)</th>
<th>D cpm (32p) of purified HgRNA in hybridization (3)</th>
<th>E % hybridization with cDNA globin (4)</th>
<th>F % hybridization with cDNA globin (5)</th>
<th>G pg globin RNA (6)</th>
<th>H pg globin RNA per pg HgRNA (7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) as described in Materials &amp; Methods</td>
<td>1,500,000</td>
<td>100%</td>
<td>220,000</td>
<td>2.3</td>
<td>220</td>
<td>40%</td>
<td>25</td>
</tr>
<tr>
<td>2) lmM UTP and no HgUTP</td>
<td>2,100,000</td>
<td>140%</td>
<td>40</td>
<td>&lt;0.01</td>
<td>20</td>
<td>1.0%</td>
<td>1</td>
</tr>
<tr>
<td>3) plus 10 µg/ml actinomycin</td>
<td>480,000</td>
<td>30%</td>
<td>43,000</td>
<td>0.4</td>
<td>63</td>
<td>9.4%</td>
<td>5.5</td>
</tr>
<tr>
<td>4) plus 1 µg/ml t-amaminitin</td>
<td>1,800,000</td>
<td>120%</td>
<td>98,000</td>
<td>1.0</td>
<td>160</td>
<td>28%</td>
<td>15</td>
</tr>
<tr>
<td>5) plus 1 µg/ml rifampicin</td>
<td>30,000</td>
<td>2%</td>
<td>4,000</td>
<td>0.04</td>
<td>24</td>
<td>1.8%</td>
<td>1.5</td>
</tr>
</tbody>
</table>

(1) These counts were consistently 2.5 x the total counts recovered from the sulfhydryl columns in all washes. DNA trapping of very short oligonucleotides may be responsible for this phenomenon. Lowering the specific activity in the synthesis reaction from 0.2Ci/mmol to 0.04 Ci/mmol eliminates this phenomenon.

(2) All hybridizations were done in duplicate.

(3) Specific activity 0.2 Ci/mmol, 30% efficiency of counting.

(4) Uncorrected cpm [32P] of samples digested with SI nuclease and TCA precipitated following a 48-hour saturation hybridization with [32P]cDNA globin, RNA in excess. The amount of cDNA (528 cpm, approximately 1 pg) included in each hybridization was determined by the average of 2 samples that were neither hybridized to globin RNA nor subjected to SI digestion prior to TCA precipitation. Combined machine background and SI background (cDNA globin undigestible by SI nuclease) of 15 cpm was determined by the average of 2 samples that were not hybridized to globin RNA but were subjected to SI digestion and TCA precipitation.

(5) Hybridization determined by cpm in column F and amount of cDNA added, after correcting for background (see footnote 4).

(6) Determined by hybridization curve of known amounts of purified mouse globin RNA included in this hybridization.

(7) Pg of globin RNA (column II) divided by pg of HgRNA (column E). N.C. indicates not calculated.

These results differ from those of Zasloff and Felsenfeld, who found inhibition of total RNA synthesis in the presence of actinomycin without a corresponding inhibition in the synthesis of globin RNA sequences as detected by
hybridization$^{13}$. Synthesis of total RNA was not affected by the presence of 1 \( \mu \text{g/ml} \) of \( \alpha \)-amanitin, nor was the synthesis of globin RNA sequences in the samples that were hybridized. Since the level of \( \alpha \)-amanitin used (1 \( \mu \text{g/ml} \)) should inhibit mammalian RNA polymerase II$^{26}$, the synthesis of globin RNA sequences does not appear to be due to endogenous RNA polymerase II activity present in the prepared chromatin. Evidence that the synthesis of globin RNA sequences is due instead to E. coli RNA polymerase comes from the assay with rifampicin. At the doses used in this study, rifampicin inhibits E. coli RNA polymerase but not any mammalian RNA polymerases$^{27}$. It can be seen that rifampicin inhibits the synthesis of total RNA by 98% and of globin RNA sequences that can be detected by hybridization by at least 90%. These results, indicating that the synthesis of globin RNA sequences from chromatin by E. coli RNA polymerase is sensitive to actinomycin and rifampicin and insensitive to \( \alpha \)-amanitin, have been reproduced in our laboratory in two other independent experiments.

Assays were performed to determine whether there was a difference in the synthesis of globin RNA sequences from induced and uninduced erythroleukemic cells. Chromatin was simultaneously prepared from induced and uninduced cells and HgRNA was synthesized in parallel in two separate reactions. The results are presented in Table II, experiment A. Synthesis of globin RNA sequences from uninduced cell chromatin was 5 to 10 fold lower than the synthesis from induced cell chromatin in this experiment. Thus, chromatin from induced and uninduced cells appears to be significantly different in its ability to direct the synthesis of globin RNA sequences by E. coli RNA polymerase.

To demonstrate that the source of globin RNA sequences detected by hybridization is not endogenous globin RNA present in induced cell chromatin, isolated uninduced cell chromatin was seeded with 175 ng of purified globin RNA (kindly provided by Dr. Stuart Orkin) prior to the synthesis of RNA. The same chromatin was also used in a parallel but separate RNA synthesis reaction without added globin. The results are presented in Table II, experiment B. There was no detectable (<1 pg) globin RNA sequences in 2 \( \mu \text{g} \) of isolated HgRNA synthesized from uninduced cell chromatin. When 175 ng of purified globin RNA was added to uninduced cell chromatin, 4.5 pg of globin RNA sequences were detected in 4.4 \( \mu \text{g} \) of purified HgRNA (sum of duplicate hybridizations, Table II, experiment B). This is 10 times less than the globin RNA sequences detected in an equivalent amount of purified HgRNA synthesized from induced cell chromatin (53 pg/4.6 \( \mu \text{g} \), sum of duplicate hybridizations, Table I). The 4.5 pg of globin RNA sequences detected are less than one part in
### Table II

Hybridization of cDNA Globin to Purified HgRNA from Erythroleukemic Chromatin

<table>
<thead>
<tr>
<th>Experiment</th>
<th>RNA synthesis reaction</th>
<th>cpm [3H] of purified HgRNA</th>
<th>calculated μg HgRNA in hybridization</th>
<th>hybridization with cDNA globin, cpm</th>
<th>% hybridization with cDNA globin</th>
<th>pg globin RNA</th>
<th>pg globin RNA per pg HgRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>induced cell chromatin</td>
<td>21,000</td>
<td>1.1</td>
<td>91</td>
<td>13%</td>
<td>23</td>
<td>2 x 10^-5</td>
</tr>
<tr>
<td></td>
<td>uninduced cell chromatin</td>
<td>31,000</td>
<td>1.6</td>
<td>37</td>
<td>3.6%</td>
<td>6</td>
<td>4 x 10^-6</td>
</tr>
<tr>
<td>B</td>
<td>uninduced cell chromatin</td>
<td>39,000</td>
<td>2.0</td>
<td>20</td>
<td>0%</td>
<td>&lt;1</td>
<td>&lt;5 x 10^-7</td>
</tr>
<tr>
<td></td>
<td>uninduced cell chromatin plus 175 ng globin RIA</td>
<td>43,000</td>
<td>2.2</td>
<td>39</td>
<td>4.2%</td>
<td>2.5</td>
<td>1 x 10^-6</td>
</tr>
</tbody>
</table>

1. Specific activity 0.4 Ci/mmol, 30% efficiency of counting.
2. See footnote 4, Table I. The amount of cDNA included in each hybridization was 617 cpm, experiment A; and 549 cpm, experiment B; both approximately 1 pg of cDNA. The combined machine and SI background was 15 cpm in experiment A and 17 cpm in experiment B.
3. See footnote 5, Table I.
4. Determined by hybridization curve of known amounts of purified mouse globin RNA included in each experiment.
5. Pg of globin RIA (column F) divided by pg of HgRNA (column C).

$10^4$ of the 175 ng of globin RNA added to the uninduced chromatin. Since 175 ng represents approximately 12 times more globin RNA than the amount expected to be present in induced cell chromatin, these results indicate that even in the presence of large amounts of globin RNA, the potential artefacts of column aggregation by HgRNA (in this case, bulk synthesized HgRNA) and RNA directed RNA synthesis by E. coli RNA polymerase are not responsible for the synthesis of globin RNA sequences from induced cell chromatin.

Tests also were carried out to demonstrate that the RIA:cDNA globin hybrids detected in these experiments contain newly synthesized HgRNA. The ability to detect the presence of HgRNA in RIA:cDNA hybrids is limited by the efficiency of the sulphydryl sepharose column in binding mercurated polynucleotides hybridized to non-mercurated polynucleotides (binding efficiency about 50%, 13, 28). In addition, loss of Hg from HgRNA might be expected during the time the HgRNA is in solution containing 0.1 M $\beta$-mercaptoethanol as well as during hybridization because of elevated temperature 23. RNA:cDNA globin hy-
Table III

Detection of HgRNA in Isolated RNA: cDNA globin Hybrids

<table>
<thead>
<tr>
<th>Hybridization Reaction</th>
<th>Control globin, no RNA (1)</th>
<th>HgRHA (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCA precipitated cpm $^{32}P$ recovered in column washes (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Column buffer + 0.5% SDS</td>
<td>34</td>
<td>810</td>
</tr>
<tr>
<td>Column buffer</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Column buffer + 0.1M $\beta$-mercaptoethanol</td>
<td>0</td>
<td>380</td>
</tr>
</tbody>
</table>

1. Control hybridization without RNA done in parallel with hybridization B.
2. HgRNA was purified from a standard RNA synthesis reaction as described in Materials and Methods. Following a 24-hour hybridization with cDNA globin in excess ($6 \times 10^5$ cpm $^{32}P$), the hybridization mixture was treated with SI nuclease to digest unhybridized cDNA. Following digestion the mixture was made 0.5% SDS, and applied to 1 ml sulfhydryl sepharose columns overlayed with 0.2 ml of Chelex 100 (this removes zinc present in the SI digestion buffer). The columns had been prewashed as described in Materials and Methods. Columns were washed with column buffer plus 0.5% SDS, column buffer, and finally column buffer plus 0.1M $\beta$-mercaptoethanol. All the washes were TCA precipitated and $^{32}P$ was counted.
3. $^{32}P$ cpm minus machine background of 15 cpm.

Hybrids were isolated and tested for the presence of HgRNA. The results presented in Table III, show that 32% (380/380+3810) of the hybridized $^{32}P$ counts bind to sulfhydryl sepharose, and are eluted with 0.1M $\beta$-mercaptoethanol, indicating the presence of HgRNA in hybrid with globin cDNA.

DISCUSSION

The results presented above demonstrate the synthesis of globin RNA sequences from chromatin isolated from DMSO induced erythroleukemic cells. The synthesis of globin RNA sequences was shown to be actinomycin sensitive, rifampicin sensitive and G-amanitin resistant. The results indicate that double stranded DNA rather than single stranded RNA is the template for synthesis and also that the polymerase involved is E. coli RNA polymerase and not the endogenous mammalian RNA polymerase II. Synthesis of globin RNA sequences was not detected in significant amounts from uninduced cell chromatin to which purified globin RNA (12 times more than the amount expected to be present in chromatin prepared from induced cells) was added. This suggests that the artefacts of aggregation of RNA in sulfhydryl column chromatography and RNA directed RNA synthesis by E. coli RNA polymerase are not responsible for the
synthesis of globin RNA sequences from induced cell chromatin.

In addition, it was shown that isolated cDNA:RNA globin hybrids bind to a sulfhydryl sepharose affinity column. This reveals the presence of mercury in the RNA and indicates that some of the hybridized RNA is newly synthesized. These results are in contrast to those of Zasloff and Felsenfeld\textsuperscript{13}, who failed to detect any (\(<1\%\)) HgRNA in their isolated hybrids. We have observed that not all batches of E. coli RNA polymerase (even from the same supplier) will synthesize globin RNA sequences. This may explain in part the differences observed between this study and previous studies of a similar nature\textsuperscript{12, 13}.

Other possible sources of difference include preparation of chromatin and conditions of RNA synthesis. Recently, other investigators have used E. coli RNA polymerase to synthesize HgRNA from chromatin and cDNA to detect the synthesis of specific RNA sequences and have also shown that isolated cDNA:RNA globin hybrids contain mercury, suggesting that the hybridized RNA sequences detected in these experiments were newly synthesized\textsuperscript{20, 30}.

The fraction of globin RNA sequences synthesized (detected by hybridization) to total RNA synthesized (determined by specific activity) in the experiments presented in Tables I and II is \(1-2 \times 10^{-5}\) part, which is about 20-40 times the proportion of DNA coding for the four mouse globin genes (\(2\alpha, 2\beta\)) in the total mouse genome (\(5 \times 10^{-7}\)). While this indicates that the RNA synthesis is not totally random from the DNA, it is approximately 100 times lower than the ratio of globin RNA to total RNA transcribed in the intact cell (\(1-3 \times 10^{-3}\) part)\textsuperscript{31}.

At present, the relationship of the in vitro synthesized globin RNA sequences to the in vivo transcript is unclear in terms of initiation, termination, and asymmetry. It is not certain whether the observed in vitro synthesized globin RNA sequences are due to initiation or elongation of pre-existing RNA chains. It is also not certain that the RNA chains synthesized in vitro are complete, since it has recently been observed that mercury may cause premature chain termination during transcription\textsuperscript{32}.

Asymmetry of transcription of single copy genes has been observed for RNA synthesis by both intact cells and isolated nuclei but not for RNA synthesis from chromatin by either homologous RNA polymerase II or E. coli RNA polymerase\textsuperscript{11, 24, 33}. The analysis of asymmetric RNA synthesis from chromatin by E. coli RNA polymerase is complicated by the fact that the polymerase can aberrantly copy endogenous chromatin RNA, synthesizing a product that is indistinguishable from RNA transcribed from the non-coding DNA strand. Although we used cation conditions which should minimize this form of aberrant tran-
scription, we still find that some RNA directed RNA synthesis is occurring. Since asymmetric RNA synthesis from chromatin by exogenous homologous RNA polymerase III has been observed, it may be possible to establish conditions for similar RNA synthesis with homologous RNA polymerase II.

Although in vitro RNA synthesis using purified nuclei is asymmetric, the complexity of the eukaryotic nucleus may prevent the in vitro manipulation of nuclear components, and thus limit understanding the function of these components in the intact cell. While reconstitution of chromatin with maintenance of its regulatory capabilities may be achievable, reconstitution of nuclei does not seem likely.

We have also performed assays to compare the synthesis of globin RNA sequences from MeSO induced and uninduced erythroleukemic cell chromatin. These results suggest that globin RNA sequences are synthesized from induced cell chromatin at a rate at least 5-10 times that of uninduced chromatin. This is in agreement with the results of earlier experiments involving RNA synthesis from chromatin (but which may have been detecting endogenous RNA) and recent experiments involving synthesis by isolated nuclei. However, these results seem to be at variance with those of experiments in which DNase digestion of chromatin has been used as a probe for transcriptional activity. A correlation has been found between the presence of cytoplasmic mRNA in intact cells and the accessibility of the corresponding coding DNA in isolated chromatin to digestion with DNase I or II. Since globin DNA is accessible to DNase II in murine erythroleukemic cells whether or not they are induced, whereas the in vitro RNA synthesis studies above suggest that little or no transcription is occurring in uninduced cells, it is possible that the chromatin structure which allows DNase digestion may not be enough to insure that transcription is occurring in vivo.

The results presented in this paper demonstrate that chromatin can be isolated from induced erythroleukemic cells in a form which allows a non-homologous prokaryotic RNA polymerase to synthesize globin RNA sequences from genes that were being transcribed in the intact cells. At least some of the complications raising questions about previous results of RNA synthesis from chromatin have been avoided in this study. This system could be of use in analyzing the regulation of gene expression in mammalian cells.

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stitute grant CA 16751 and National Institute of Child Health and Human Development grants HD 04807 and HD 06276.

REFERENCES

1. The abbreviations used are: DMSO, dimethylsulfoxide; HgRNA, mercurated RNA; non-HgRNA, non-mercurated RNA; HgtRNA, mercurated transfer RNA; HgUTP, 5-mercuriuridine triphosphate, TCA, trichloroacetic acid.