Release of a globin gene enriched chromatin fraction from chicken erythrocyte nuclei following DNase II digestion

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ABSTRACT:
Mild digestion of chicken erythrocyte nuclei with deoxyribonuclease II results in the release of a chromatin fraction which is 4- to 13-fold enriched for the globin coding sequences when compared to total chicken DNA. The remaining nuclear pellet is depleted in these sequences. A maximum of 25% of the globin genes have been recovered in the released fraction. The addition of 5 mM sodium butyrate to the digestion buffer is required to obtain reproducible globin gene enrichment. The released fraction contains equimolar amounts of the four core histones and a subset of the nonhistone chromosomal proteins. The globin genes are released as large chromatin fragments which exceed the 1.6 kilobase size of the transcribed portion of the gene.

INTRODUCTION

The factors which enable some genes in a cell to be a template for DNA dependent RNA polymerase II are not yet known. One approach to studying the transcribed gene would be to separate the transcriptionally active from non-transcribing chromatin and study the differences. For many years investigators have been attempting to fractionate chromatin by a variety of procedures involving fragmentation of the chromatin by sonication or mild nuclease digestion followed by fractionation based on differential solubility, sedimentation, or chromatographic properties. Recently, several laboratories have reported isolation of a transcriptionally active chromatin fraction using a specific gene probe as a marker for this fraction (1,2,3,4,5).

One method of fractionation was based on the suggestion that mild DNase II digestion followed by Mg++ precipitation of the bulk of the chromatin might lead to the release of transcriptionally active chromatin (6). Using this procedure, the fractionation of globin genes from mouse erythroleukemic cell and chicken reticulocyte chromatin has been reported (1,2,3). On the other hand there is some evidence that active gene fractionation following DNase II digestion is not always found using this fractionation
procedure, since at least one laboratory failed to obtain globin gene fractionation from Friend cell chromatin (7). Furthermore, except in one case where good yields of total globin gene in the active fraction are reported (1), yields are either low or unreported. The failure to obtain reproducible globin gene fractionation suggested that there might be subtle differences among the methods of chromatin isolation and/or digestion which enable some laboratories to obtain fractionation while others cannot.

It has been shown that DNase II digestion-Mg++ precipitation of chromatin releases a hyperacetylated chromatin fraction (8,9). Perhaps fractionation depends upon hyperacetylation, and difficulty in obtaining transcriptionally active chromatin is caused by the removal of the acetyl groups by the enzyme histone deacetylase. A potent inhibitor of histone deacetylase, sodium butyrate (10,11,12,13), was added to determine if it had any effect on fractionation. Using a fractionation procedure which differs slightly from the one employed in the earlier studies, the addition of sodium butyrate was found to be necessary for the reproducible isolation of a globin gene enriched chromatin fraction from chicken erythrocyte nuclei following DNase II digestion. The level of globin gene enrichment obtained by this procedure is two to five times higher than that reported in previous studies and the globin enriched chromatin was obtained in sufficient yield for biochemical characterization.

MATERIALS AND METHODS

Preparation of Erythrocyte Nuclei. Blood was taken from mature white leghorn hens immediately prior to use. The blood was drawn into syringes containing 200 mM EDTA pH 7.0 to prevent clotting (final EDTA concentration of approximately 5 mM). It was chilled on ice and the preparation was kept cold until the nuclease digestion. Erythrocytes were collected by centrifugation at 1500 x g for 5 min. The cells were washed three times in 100 mM KCl, 10 mM Tris (HCl) pH 8.0, 5 mM MgCl2, 5 mM sodium butyrate pH 7.0, 0.1 mM PMSF (KTMB buffer-final pH 7.9) and were lysed in KTMB buffer plus 0.2% Triton X-100. The nuclei were collected by centrifugation at 2500 x g for 5 min. and were washed several times in KTMB plus Triton until they were white in color. They were sedimented through KTMB buffered 2.2 M sucrose at 10,000 x g for 60 min. and then washed twice in KTMB buffer and stored overnight. Prior to digestion, the nuclei were washed twice in 25 mM KCl, 10 mM Tris (HCl) pH 7.4, 2 mM MgCl2, 5 mM sodium butyrate pH 7.0 and diluted to a concentration of 5 mg DNA/ml (OD260 = 1 mg/ml). This nuclear preparation
procedure apparently washes out all endogenous nucleases and proteases since no alteration in the electrophoretic mobility of the nucleic acid or chromosomal proteins was observed when the nuclei were incubated at 37° for 45 min.

Digestion of Nuclei and Fractionation of Chromatin. Erythrocyte nuclei were digested with spleen acid DNase (Worthington) according to the digestion scheme shown in Figure 1. Many lots of commercially prepared enzyme were found to be contaminated with proteases which degraded chromosomal proteins under the digestion conditions needed for globin gene release. Only lots of enzyme which did not produce any alteration in the electrophoretic mobility of chromosomal proteins in SDS-polyacrylamide gels were used in these experiments. Since some lots of enzyme consistently produced better yields than other lots, all the enzyme preparations used were not identical.

Nuclei were digested with 150-600 units DNase/mg DNA at 37° for 45-90 min (enzyme units as described by Worthington). The high enzyme concentrations are necessary because the neutral pH and 2 mM MgCl₂ required to maintain intact nuclei are nonoptimal enzyme conditions. The divalent cation concentration has been reported to differentiate between soluble transcriptionally active and insoluble inactive chromatin (6). Following the digestion, the nuclei were chilled on ice for 10 min and then sedimented at 16,000 x g for 10 min. The soluble chromatin is called the released fraction. In some cases the pellet was washed in 10 mM Tris(HCl) pH 8.0, 2 mM MgCl₂ and additional soluble chromatin was obtained when the nuclei were resedimented. The two released fractions (called released fraction₁ and released fraction₂) were pooled in some experiments and analyzed separately in others. The nuclei, together with any insoluble chromatin, is called the nuclear pellet fraction.

Purification of DNA. Chromatin fractions were adjusted to 0.5 M NaCl, 0.2% SDS, and the chromosomal proteins digested with 0.1 mg/ml proteinase K (Beckman) at 37° overnight. The DNA was sonicated and extracted twice with an equal volume of phenol and then several times with chloroform:iso-amyl alcohol (21:1). The DNA samples were incubated in 0.3 N NaOH at 37° overnight to degrade any mRNA, neutralized, precipitated from solution with ethanol several times, and resuspended in 0.1 mM EDTA pH 7.0.

Purification of Chicken Globin mRNA. Chicken globin mRNA was isolated by a procedure similar to the one described by Longacre and Rutter (14). Reticulocytes were obtained from phenylhydrazine treated white leghorn hens. The cells were washed and subjected to hypotonic lysis and the nuclei removed by sedimentation. Polysomes were collected by centrifugation and were
dissociated into ribosomes and ribonucleoprotein particles with EDTA. These components were separated on a 15 to 30% sucrose gradient (15). The 13S globin ribonucleoprotein particle peak was collected and the proteins extracted with phenol-chloroform-isoamyl alcohol. The polyadenylated RNA was isolated by fractionating the RNA on oligo-dT cellulose. In the last step of the purification, the mRNA was separated on a 15 to 30% sucrose gradient and a 10S peak of globin mRNA was collected.

**Synthesis of [³H]-cDNA Probe.** [³H]-cDNA probe was synthesized from 10S polyadenylated globin mRNA using the enzyme RNA-dependent DNA polymerase (reverse transcriptase). The reaction mixture contained 50 mM Tris(HCl) pH 8.3, 50 mM KCl, 10 mM MgCl₂, 5 mM dithiothreitol, 4 mM sodium pyrophosphate, 5 µg/ml oligo dT₁₂₋₁₈ (Collaborative Research), 0.3 mg/ml actinomycin D (Calbiochem), 0.8 mM dATP, dGTP, and dTTP, 0.2 mM [³H]-dCTP (New England Nuclear-25 Ci/mmol), 20 µg/ml globin mRNA, and 50 units/ml reverse transcriptase (supplied by Dr. J. Beard through the Program Resources Branch, National Cancer Institute). An additional 20 mM potassium phosphate pH 7.2, 0.2 mM dithiothreitol, 0.02% Triton X-100, 5% glycerol were added to the reaction mixture with the addition of the enzyme. The reaction was incubated at 37° for 60 min. The reaction was terminated, the mRNA degraded, and the probe purified as described in Zasloff and Felsenfeld (16). The probe was concentrated by extraction with sec-butanol followed by two extractions with chloroform. The specific activity of the cDNA was 1.2 x 10⁴ cpm/ng. Approximately 85% of the cDNA was competent to hybridize to globin mRNA and only 5% self-annealed under the hybridization conditions used.

**Determination of Globin Gene Content.** The globin gene content of the various DNA samples was determined by cDNA excess hybridization with at least a 10-fold excess of globin cDNA sequences over the number of globin sequences in the chicken DNA samples. A 20 µl hybridization reaction contained 0.5 ng [³H]-cDNA and, for unenriched DNA samples, 20-60 µg chicken DNA. Calf thymus DNA was added so that the total amount of DNA in each sample was approximately 100 µg. Less DNA was analyzed from the globin enriched samples but the amount of cDNA hybridized was in the same range as the other samples. The hybridizations were done in a buffer containing 200 mM sodium phosphate pH 6.8, 0.5% SDS, 1 mM EDTA pH 7.0. The samples were sealed in glass capillaries and were denatured at 107° for 10 min and reannealed at 68° for 15-18 hr. The amount of [³H]-globin cDNA in hybrid was determined following digestion of single-stranded DNA with S1 nuclease. The reannealed samples were expelled into 2 ml 30 mM sodium acetate pH 4.6, 100 mM NaCl, 1 mM ZnSO₄.
20 μg/ml denatured calf thymus DNA and digested with 100 units [as determined by Vogt (17)] SI nuclease (P-L Biochemicals) at 45° for 45 min. Undigested double-stranded DNA was precipitated with TCA and trapped on glass fiber filters. The amount of radioactivity on each filter was assayed by liquid scintillation counting. The globin gene content of a DNA sample was determined by plotting the mass of chicken DNA in the reaction against the cpm of \( ^3\)H\text{-cDNA} hybridized. The ratio between the slopes of a DNA sample and a sonicated chicken DNA control gives the relative globin gene content. The genomic abundance of globin gene on this scale is 1.0.

RESULTS

Chromatin Fractionation in the Presence and Absence of Sodium Butyrate.

In order to test the hypothesis that active gene fractionation might be intimately related to hyperacetylation, two batches of chicken erythrocyte nuclei were prepared under identical conditions except for the addition of 5 mM sodium butyrate to the buffers of one of the samples. The nuclei were digested with 400 units DNase II/mg DNA at 37° for 45 min according to the digestion scheme shown in Figure 1. During the digestion chromatin was released from the nuclei of both digests and remained soluble when the digests were chilled and the nuclei plus insoluble chromatin collected by

![Diagram of chromatin fractionation](image)

**Figure 1:** Erythrocyte nuclei digestion procedure.
centrifugation. The DNAs from the soluble released chromatin fractions and
the insoluble nuclear pellet fractions were isolated and their globin gene
content determined by cDNA excess hybridization. The data shown in Table 1
indicate that both fractions from the sample prepared without sodium butyrate
have approximately the genomic abundance of globin. By contrast, the re-
leased fraction from the sodium butyrate treated sample has almost five times
the genomic globin gene content while the pellet fraction is depleted in
globin sequences.

Experiments were done to determine if the sodium butyrate was critical
to the nuclear isolation or the digestion step of the fractionation. Table 1
shows an experiment in which the butyrate was removed from the isolation buffer.
It is clear that the butyrate is not required during the nuclear isolation
in order to obtain globin gene fractionation. Since globin gene fractionation
is sodium butyrate dependent and since the butyrate is not required during the
nuclear isolation, it must function during the digestion. However, it was not
possible to demonstrate this point conclusively by having butyrate only in the
isolation buffer. A sample isolated in the presence of butyrate but digested
without it had a pellet fraction which was depleted of globin genes (data not
shown). Presumably there was sufficient butyrate remaining in the nuclei
despite repeated washing to maintain the nuclei in a state in which genes could
be fractionated. It should be noted that small amounts of butyrate are suffi-
cient to inhibit histone deacetylases (10, 11, 12, 13).

Table 1: Globin Gene Content of Sodium Butyrate Treated Samples.

<table>
<thead>
<tr>
<th>sodium butyrate</th>
<th>globin gene content</th>
</tr>
</thead>
<tbody>
<tr>
<td>isolation</td>
<td>digestion</td>
</tr>
<tr>
<td></td>
<td>released fraction</td>
</tr>
<tr>
<td></td>
<td>pellet fraction</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>+</td>
<td>+</td>
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<td>-</td>
<td>+</td>
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<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Nuclear isolations and DNase digestions with and without 5 mM sodium butyrate
were done as described in the Materials and Methods section. Samples not
fully treated with butyrate were run in parallel with the fully treated control
shown in the line immediately below it. The globin gene content of the
chromatin fractions was determined by cDNA excess hybridization.
These results indicate that in this experimental system sodium butyrate can maintain the chromatin in a state susceptible to fractionation. Additional experiments were done in the presence of sodium butyrate to determine if fractionation is reproducible and if a sufficiently high yield of globin enriched chromatin could be obtained for further studies. Another globin gene fractionation experiment is shown in Figure 2 and Table 2. In this case, nuclei were digested with 600 units DNase II/mg DNA for 45 min. Slightly less than 5% of the chromatin was released from the nuclei by the digestion while less than 0.5% of the chromatin was rendered acid soluble. The cDNA excess hybridization curve (Figure 2) shows that the DNA in the released fraction was enriched 7.1-fold in globin genes, while the nuclear pellet had only 0.44 the normal content. In other similar experiments, enrichments of 4- to 13-fold were found. Failure to obtain enrichment occurred in only one of eight experiments. The maximum globin gene depletion obtained in the pellet fraction was about 40% of the original content. In the experiment shown in Table 2, it is possible to account for 69% of the

![Figure 2: cDNA excess hybridization of DNA from the chromatin fractions. Erythrocyte nuclei were digested with 600 units DNase II/mg DNA for 45 min. The digestion, fractionation, extraction, and hybridization conditions are described in Materials and Methods. Released fraction (o), pellet fraction (a), sonicated DNA control (e). Each point is the average of two determinations. The DNA samples were analyzed in two independent experiments with identical results.](image-url)
Table 2: Percentage of Globin Gene Recovered in the Chromatin Fractions.

<table>
<thead>
<tr>
<th>fraction</th>
<th>globin gene content</th>
<th>percent DNA</th>
<th>percent globin gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>unfractionated</td>
<td>1.0</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>released₁</td>
<td>7.1</td>
<td>3.2</td>
<td>23</td>
</tr>
<tr>
<td>released₂</td>
<td>3.6</td>
<td>0.8</td>
<td>4</td>
</tr>
<tr>
<td>pellet</td>
<td>0.44</td>
<td>96</td>
<td>42</td>
</tr>
</tbody>
</table>

This is a tabulation of the data from the experiment shown in Figure 2. The globin gene content was determined from the cDNA excess hybridization curves. The percent DNA in each fraction was determined from the optical density of the chromatin samples immediately following the digestion. The percent gene recovered is the product of the two numbers.

The original globin gene content of the nuclei with 27% of the genes recovered in the released fractions. This percentage of globin genes in the released fractions was the highest value obtained and it came from the most extensively digested sample. Table 3 summarizes the globin gene recoveries from the four experiments carried out in sufficient detail to permit a complete accounting. In these experiments which involve different extents of digestion, the average overall recovery of globin genes was 62%, and an average of 14% was recovered in the enriched fraction.

The pH of the digest is a second critical factor in obtaining globin

Table 3: Average Percent Recovery of Globin Genes.

<table>
<thead>
<tr>
<th>released fraction</th>
<th>pellet fraction</th>
<th>total recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>36</td>
<td>49</td>
</tr>
<tr>
<td>7</td>
<td>63</td>
<td>70</td>
</tr>
<tr>
<td>27</td>
<td>42</td>
<td>69</td>
</tr>
<tr>
<td>9</td>
<td>49</td>
<td>58</td>
</tr>
</tbody>
</table>

This table summarizes the globin gene recoveries from four experiments. Erythrocyte nuclei were digested with 200-600 units DNase II/mg DNA for 45-90 min. The fractionation, extraction, and hybridization conditions are described in the Materials and Methods section. The results were calculated similarly to those in Table 2.
gene fractionation following DNase II digestion. When the pH of a portion of a digest was raised to 8.5 prior to fractionation to prevent additional digestion of the sample, both the pellet and released chromatin fractions had the genomic abundance of globin. In the control experiment, a second aliquot of the same digest fractionated at pH 7.4 showed a released fraction enrichment of 12.8-fold and pellet depletion of 0.4-fold. This loss of fractionation does not simply reflect a pH dependent precipitation since the globin genes did not precipitate when the pH of a globin enriched released fraction was raised.

Characterization of the Released Chromatin Fraction.

A sufficient amount of the globin enriched chromatin was obtained to permit analysis of its protein composition. Figure 3 shows an SDS polyacrylamide gel of the total nuclear proteins after DNase II digestion, and of the proteins from a released chromatin fraction which was enriched 7-fold for globin sequences. The released chromatin sample was overloaded to reveal small amounts of nonhistone protein components. When the sample is run at a lower concentration, the histones appear to be present in equimolar amounts. In this experiment, the amounts of histones H1 and H5 in the released fraction are similar to unfractionated chromatin. However, at an earlier digestion time the relative amount of these histones was somewhat reduced. In contrast to

![Figure 3: SDS polyacrylamide gel of the proteins from the released chromatin fraction. The chromatin proteins were solubilized in 1% SDS and run on an SDS polyacrylamide gel according to the procedure described in Weintraub and Van Lente (18). The positions of the histones are indicated on the figure. Protein samples were from the experiment shown in Figure 2 and Table 2. (left panel) Released fraction (15 μg). (right panel) Total digest (40 μg).](image-url)
the pattern of unfractionated material, the released fraction has only a subset of the total nuclear nonhistone chromosomal proteins. Additional experiments must be done to determine which of these nonhistone chromosomal proteins are tightly associated with high molecular weight DNA and therefore might be a constituent of the globin enriched chromatin. Experiments using urea-acetic acid polyacrylamide gels to resolve the acetylated forms of histones H3 and H4 suggest that H4 and perhaps H3 in the released fraction are shifted toward slightly higher acetylation states while the histones of the pellet fraction appear indistinguishable from those of undigested chromatin (Figure 4). These results are in accord with those of Davie and Candido (9) who found an increased level of H4 acetylation in DNase II solubilized chromatin.

The size of the DNA in the released fractions has been examined by electrophoresis in acrylamide and agarose gels. A range of large fragments from 0.5 to 15 kilobases was found. The sizes of the globin gene fragments were determined by the Southern transfer technique (22). Figure 5 shows an autoradiogram of a transfer which was hybridized to a fragment from pHb 1001, a cloned beta-globin cDNA probe (24). The beta-globin genes in the released fraction were found in fragments from 2 to 7 kilobases in length. Under these conditions, no fragments of discrete size are seen. In a subsequent

![Figure 4: Densitometry tracings of the proteins from the chromatin fractions analyzed on urea-acetic acid polyacrylamide gels. The proteins were acid extracted and run on a urea-acetic acid polyacrylamide gel by a modification of the procedures of Panyim and Chalkley (19) and Alfageme, Zweidler, Mahowald, and Cohen (20) as described in Tack (21). A negative from the acrylamide gel was scanned with a Joyce-Loebl microdensitometer. The positions of the histones are indicated on the figure. The subscripts indicate the number of acetylated groups. 1. Pellet fraction (60 ug). 2. Released fraction (15 ug). 3. Undigested control (40 ug).](image-url)
Figure 5: Southern transfer of DNA from the released fraction. (left panel) DNA from the released fraction (3 μg) was run on a 1% agarose gel, transferred to nitrocellulose paper and probed with [\(^{32}P\)]-labelled Hsp I fragment from pHb 1001 (kindly provided by Dr. G. Ginder). The average molecular weight of the hybridizing DNA fragments is 4 kb. (right panel) \(\lambda\) Hind III digest (New England BioLabs) from the agarose gel. The procedures for this experiment have been described in Ginder, Wood, and Felsenfeld (23).

experiment, the mean size of the globin gene fragments was found to vary depending upon the extent of digestion. This observation suggests that the failure to recover 100% of the globin genes following DNase II digestion which was seen in Table 3 results from the degradation of the genes into small non-hybridizable fragments. Both the native and denatured DNA had similar transfer patterns indicating that the globin enriched DNA was not extensively nicked during the digestion. An autoradiogram of a transfer from an identical agarose gel hybridized to a cDNA probe made from 10S polyadenylated chicken globin mRNA which hybridizes to several globin genes in addition to the beta-globin gene revealed a pattern of globin gene fragments similar to that obtained with the cloned plasmid probe (data not shown). The similarity between the two transfer patterns confirms that the cDNA titrations shown
earlier are detecting globin gene sequences.

DISCUSSION

Using the experimental procedure described in this paper, it is possible to obtain reproducibly a chromatin fraction from chicken erythrocyte nuclei which is 4- to 13-fold enriched for the globin coding sequences. The reasons for this preferential release of the globin genes from nuclei by DNase II are not known. It is assumed that a combination of the globin chromatin structure and the ionic conditions employed in these experiments render the globin coding regions preferentially excisable and/or soluble and that the structural differences between the globin coding regions and bulk chromatin reflect the transcription of these genes at an earlier time in the history of the cell.

There is considerable data which suggests that the structure of transcriptionally active chromatin is more open than in active chromatin and thus would be more accessible to nucleases (recently reviewed in Mathis, Oudet, and Chambon (25)). The preferential sensitivity of the globin genes from chicken erythrocytes to DNase I is well documented (26, 27). It also appears that hyper-acetylated chromatin fragments are more soluble in the presence of Mg\(^{++}\) than control chromatin (28).

The initial assumption was that globin gene fractionation might be related to histone acetylation and it has been demonstrated that sodium butyrate, a known inhibitor of histone deacetylase (10, 11, 12, 13), has a pronounced effect on fractionation. Other laboratories have reported globin gene fractionation from erythroid cells using a different procedure without sodium butyrate (1, 2, 3) though at least one group failed to obtain globin gene fractionation by the same procedure (7). All of these results can perhaps be understood in terms of the properties of the histone deacetylase. This chromatin-bound enzyme is inactive at low temperatures but rapidly de-acetylates histones at 37\(^\circ\) unless inactivated by an inhibitor such as sodium butyrate (21). Previous studies have used short digestion times at room temperature in acetate buffer. Although acetate is an 8-fold weaker inhibitor of histone deacetylase than butyrate (13), it should inhibit the enzyme to some extent during the critical digestion phase. However, none of the previous studies included acetate in the steps prior to the nuclease digestion. Thus, the variability in yield and enrichment of the released fraction in those experiments may reflect the use of conditions which only marginally inhibit deacetylation. A previous study found some histone deacetylation when nuclei were incubated under micrococcal nuclease and DNase I digestion.
They found the addition of sodium butyrate prevented this deacetylation. It should be noted that the presence of polyamines or divalent cations, which are also weaker inhibitors of histone deacetylase (21), might also affect the outcome of the fractionation procedure.

The assumption that sodium butyrate effects fractionation by an inhibition of histone deacetylase has not been proved. It is possible that the butyrate may facilitate fractionation in some other way. Whatever its mechanism of action, this compound appears to be responsible for the 2- to 5-fold higher levels of globin gene enrichment and the reproducible release of a globin enriched chromatin fraction which have been obtained in this study.

Sufficient globin enriched chromatin was obtained for a biochemical characterization of this fraction. The avian globin genes are known to be organized in nucleosome-sized particles like the bulk of the chromatin (30). As might be expected from a nucleosomal type of organization, the SDS-polyacrylamide gel of the released chromatin proteins shows approximately equimolar ratios of the four core histones. Urea-acetic acid polyacrylamide gels of these proteins suggest that histone H4 and perhaps H3 are hyperacetylated. The released chromatin fraction is also enriched in some of the nonhistone chromosomal proteins. The electrophoretic mobility of several of these bands suggests that they may be HMG 1, HMG 14, and histone deacetylase. However, additional experiments will be necessary to identify components unequivocally, and to determine which ones are actually associated with the released DNA. Although previous studies characterizing the DNase II solubilized chromatin fraction have not demonstrated gene enrichment in the released fraction, the results obtained in this study are in general agreement with earlier observations. These studies found that the released fraction was composed of the four core histones but was depleted in histone H1 in some cases (6,31, 32). The preferentially solubilized fraction was found to be enriched in hyperacetylated-histones (8, 9) and in HMGs 1 and 2 but not 14 and 17 (33). This last observation is surprising since chicken erythrocyte mononucleosomes associated with HMG 14 and 17 are enriched for globin genes (34, 35) and actively transcribed monomers stripped of their HMG proteins can be purified by their ability to bind to HMG 14 and 17 (36). In contrast to earlier studies which found that DNase II rapidly generated slightly larger than monomer-sized nucleoprotein particles (31, 37), the globin enriched chromatin in this study is released as large fragments. These large fragments may result from the presence of hypersensitive DNase II cleavage sites around the globin genes analogous to the hypersensitive DNase I
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sites (27). A more extensive digestion might result in the appearance of a ladder of DNA fragments containing the globin genes similar to that seen with more extensively digested bulk chromatin (38). Since globin coding DNA is released as fragments larger than the 1.6 kilobases which code for the beta-globin message (23), flanking sequences are released along with the coding sequences.

This paper presents a procedure for reproducibly obtaining the release of a globin enriched chromatin fraction from nucleated chicken erythrocytes. Since this procedure can release a significant proportion of the globin genes as large chromatin fragments, it should be useful for obtaining a globin enriched chromatin fraction for study of monomers as well as higher order structure.

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