Chromatin structure of erythroid-specific genes of immature and mature chicken erythrocytes

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The β-globin and histone H5 genes are transcriptionally active in immature chicken erythrocytes and potentially active in mature erythrocytes. In both immature and mature erythrocytes, the majority of these erythroid-specific gene sequences are located in two chromatin fractions: the low-salt-insoluble residual nuclear material and the 0.15 M-NaCl-soluble oligo- and poly-nucleosomes. These salt-soluble chromatin fragments are enriched in hyperacetylated species of H4 and H2B, ubiquitinated and polyubiquitinated species of H2A and H2B and are depleted of linker histones H1 and H5. The competent, transcriptionally inactive embryonic ε-globin gene, which is part of the DNAse I-sensitive β-globin domain, is highly enriched in the 0.15 M-NaCl-soluble polynucleosome fraction but not in the insoluble nuclear material. The repressed vitellogenin gene shows no enrichment in either of these fractions. These results suggest that only those genes that are expressed or have the potential for expression are enriched in the low-salt-insoluble nuclear material of immature or mature erythrocytes. The enrichment of active genes in the low-salt-insoluble residual nuclear material of immature erythrocytes is not dependent on on-going transcription, the presence of RNA or changes in the amount of acetylated histone species. Our results are consistent with the hypothesis that active and potentially active genes are insoluble because of the presence of preinitiation transcription complexes.

INTRODUCTION

Although most active genes are packaged into nucleosomes, they are organized into a less condensed chromatin structure than inactive genes. This is reflected by their increased susceptibility to digestion by various nucleases, especially DNAase I. Although the biochemical basis for the difference between expressed and repressed gene chromatin has been extensively investigated, a complete description of these two entities has not been achieved (for reviews see Reeves, 1984; Gross & Garrard, 1987). Analysis of chromatin regions separated by salt extraction has been an informative approach to determine the composition of transcriptionally active chromatin. Transcriptionally active gene chromatin has a soluble and/or insoluble nature, that is, active genes are located in two types of chromatin fragments, those that are soluble in 0.15 M-NaCl and/or 2 mM-MgCl₂ and those that co-fractionate with the insoluble residual nuclear material (Cohen & Sheffery, 1985; Rose & Garrard, 1984; Strätling et al., 1986; Strätling, 1987; Ridsdale & Davie, 1987; Zhang & Nelson, 1988; Ridsdale et al., 1988; Nickel & Davie, 1989a).

The nature of the insoluble transcriptionally active chromatin is unknown. It has been suggested that transcriptionally active genes are insoluble because of the presence of the transcription complex (Roberge et al., 1988). Another explanation is that the expressed gene sequences are attached to the internal nuclear matrix (for review see W. G. Nelson et al., 1986). It should be noted that the immature and mature chicken erythrocytes lack an internal nuclear matrix (Lafond & Woodcock, 1983; Lafond et al., 1983).

The chicken erythrocyte is a popular system to study histone modifications and the structure of transcriptionally active and potentially active gene chromatin. Although there is general agreement that the β-globin gene is expressed in immature erythrocytes, there are conflicting reports as to whether the β-globin gene is expressed in mature chicken erythrocytes. Gariglio et al. (1981) reported that, although RNA polymerase II molecules are ‘poised’ at the 5′ end of the β-globin gene, the gene is not expressed in mature cells. In contrast, Affolter et al. (1987) demonstrated that the β-globin and histone H5 genes are transcribed in mature erythrocytes, with the transcriptional activity of the histone H5 gene in mature cells being comparable to that of immature cells.

We have demonstrated that the 0.15 M-NaCl-soluble polynucleosomes of mature chicken erythrocytes are highly enriched in DNAase I-sensitive, β-globin, histone H5 and ε-globin gene sequences (Ridsdale & Davie, 1987; Ridsdale et al., 1988). The ε-globin gene, which is no longer expressed in mature or immature erythrocytes and is part of the DNAase I-sensitive, β-globin domain (Stalder et al., 1980; Wood & Felsenfeld, 1982), is classified as competent chromatin. It is not known whether transcriptionally active, potentially active and competent chicken erythroid genes of mature and immature erythrocytes are enriched in the low-salt-insoluble nuclear material. To address this question, we examined the partitioning of erythroid-specific (ε-globin, β-globin and histone H5) genes among various fractions of

Abbreviations used: DRB, 5,6-dichloro-1-β-d-ribofuranosylbenzimidazole; PMSF, phenylmethanesulphonyl fluoride; u, ubiquitinated; MHS, micrococcal nuclease hypersensitive site.
immature and mature chicken erythrocyte chromatin. In addition, we analysed the histone composition of the chromatin fractions of immature chicken erythrocytes. We found that the β-globin and histone H5 genes, but not the competent ε-globin gene, are enriched in the low-salt-insoluble nuclear material of mature and immature erythrocytes. The selective partitioning of the β-globin and histone H5 genes with the insoluble nuclear material was not dependent on on-going transcription, the presence of RNA or the amount of acetylated histone species. Our results are consistent with the idea that the association of a preinitiation transcription complex with β-globin and histone H5 gene chromatin accounts for the insolubility of these genes. In immature erythrocytes, the 0.15 M-NaCl-soluble oligo- and poly-nucleosomes, which are enriched in active and competent genes and depleted in repressed genes, are enriched in modified histone species, including hyperacetylated species of histones H2B and H4, ubiquitinized histones H2A and H2B and polyubiquitinated species of histone H2A and depleted in linker histones, H1 and H5.

MATERIALS AND METHODS

Isolation of immature and mature chicken erythrocytes

To obtain immature chicken erythrocytes [40% early and mid-polychromatic erythrocytes and 55% late polychromatic erythrocytes (Williams, 1972)], adult White Leghorn chickens were treated with phenylhydrazine hydrochloride (Ferenz & Nelson, 1985). Red blood cells from treated and untreated chickens were collected in 75 mM-NaCl/25 mM-EDTA/30 mM-sodium butyrate (except for cells incubated without butyrate), washed of the buffy coat and stored at −70°C. For some experiments, immature erythrocytes were incubated at 37°C for 60 min in Swim's S-77 medium with or without 10 mM-sodium butyrate, or the cells were incubated at 37°C for 90 min in Swim's S-77 with or without the transcription inhibitors actinomycin D (0.04 μg/ml) and/or 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) (75 μM) (Ericsson et al., 1986).

Fractionation of chromatin fragments

Nuclei were isolated and digested as described previously (Ridsdale & Davie, 1987), except that 10 mM-iodoacetamide was added to the RSB buffer. Digested nuclei (fraction T) were resuspended in an equal volume of 10 mM-EDTA (pH 7.5)/1 mM-PMSE and left on ice for at least 30 min to release chromatin fragments into solution. The soluble (S1) and insoluble (P0 or low-salt-insoluble nuclear material) fractions were separated by centrifugation at 12000 g for 10 min. NaCl was added to the S0 fraction to a final concentration of 0.15 M and after centrifugation at 12000 g for 10 min, an insoluble (P150) and a soluble fraction (S150) were obtained. The S150 fraction was applied on to a Bio-Gel A-5m column to produce four fractions F1, F2, F3, and F4 (Ridsdale & Davie, 1987). RNAase digestion of the F0 fraction of immature erythrocytes was done in 10 mM-EDTA (pH 7.5)/1 mM-PMSE by incubation at 37°C for 2 h with 200 units of RNAase T1/ml and 200 μg of RNAase A/ml. After centrifugation at 12000 g for 10 min, an insoluble fraction (P200) and a soluble fraction were obtained. A mock digestion was done under the same conditions but RNAases were omitted. This produced the insoluble fraction P0.

DNA preparation and DNA hybridization

The percentage of DNA in each fraction was determined by diphenylamine assay (Giles & Myers, 1965). Chromatin samples were made up to 2.2% sodium N-dodecylsarcosine/0.16 M-NaCl/10 mM-EDTA/0.5 mg of Pronase/ml and incubated at 37°C overnight. Afterwards they were digested with 100 units of RNAase T1/ml and 100 μg of RNAase A/ml, extracted with phenol/chloroform and ethanol-precipitated. DNA was run on 1% agarose gels containing 1 μg of ethidium bromide/ml, transferred on to nitrocellulose and hybridized to radiolabelled probes as described (Thomas, 1980). Washes were done as previously described (Maniatis et al., 1982). Quantification of DNA sequences was done by slot blot analysis. The DNA was made to 0.3 M-NaOH, incubated at 37°C for 30 min, neutralized by the addition of an equal volume of 2 M-ammonium acetate, pH 7.0, and various amounts were applied on to nitrocellulose using a Schleicher and Schuell slot blotting manifold. Each slot was rinsed with 10× SSC (20× SSC = 3 M-NaCl/0.3 M-sodium citrate). After hybridization to radiolabelled probes (Thomas, 1980), the autoradiograms were scanned with a densitometer. The relation between the signal and the amount of DNA loaded was linear, showing that the intensity of the signal was directly proportional to the amount of hybridizable DNA sequence. The intensity of the hybridization signal per μg of DNA was determined for DNA which was isolated from total unfractonated chromatin (T) and the chromatin fractions. The values obtained for the DNA of each chromatin fraction were divided by the value for total DNA, determining the partitioning ratio of DNA sequences among the different chromatin fractions. If the partitioning ratio was >1, the analysed DNA sequence was enriched in that chromatin fraction, but if the partitioning ratio was <1, the analysed DNA sequence was depleted. The percentage of a DNA sequence in a chromatin fraction was obtained by multiplying the partitioning ratio of a sequence by the percentage of total DNA in that fraction (this value was determined by diphenylamine assay). The cloned DNA probes used were: pCBG14.4, an unique intronic sequence of the adult β-globin gene; pCBG18.7, an embryonic ε-globin sequence (Villepontaine et al., 1982); pChV2.5B/H, which contains the gene coding for histone H5 and some flanking sequences on both sides (Ruiz-Carrillo et al., 1983); and pVTG412, which recognizes the 5′ region of the vitellogenin gene (Burch & Weintraub, 1983).

Protein electrophoresis and Western blotting

Chromatin fractions were extracted with 0.2 M-H2SO4. After centrifugation at 12000 g for 10 min, the supernatants were dialysed against 0.1 M-acetic acid and twice against water. Polycrylamide-gel electrophoresis was performed as described (Davie, 1982). Ubiquitin protein conjugates were detected by the use of a polyclonal anti-ubiquitin antibody in Western-blotting experiments (Nickel et al., 1987).

RESULTS

Transcriptionally active and potentially active genes are preferentially located in the low-salt-insoluble residual nuclear material and 0.15 M-NaCl-soluble oligo- and poly-nucleosomes

Mature and immature chicken erythrocyte nuclei were
incubated with micrococcal nuclease, and the fragmented chromatin was fractionated by a low-salt procedure as described in the Materials and methods section. The repressed vitellogenin and competent \( \beta \)-globin DNA sequences show a typical nucleosomal ladder like that of bulk DNA (Fig. 1). In contrast, the histone H5 and \( \beta \)-globin DNA sequences, which are active in immature erythrocytes and potentially active in mature cells, exhibit a non-discrete continuum of DNA lengths (Fig. 1), which is typical of genes that are expressed or have the potential for expression (Cohen & Sheffery, 1985).

For both immature and mature erythroid chromatin, the histone H5 and \( \beta \)-globin genes are enriched in fractions \( P_{150} \) (the low-salt-insoluble nuclear material) and \( S_{150} \), the 0.15 M-NaCl-soluble chromatin (Fig. 1 and Table 1). The \( \beta \)-globin and histone H5 DNA sequences are enriched to similar extents in both mature and immature erythrocyte insoluble nuclear material (\( P_{150} \)), with an enrichment of 4.7–5.6-fold. However, around 10% of the DNA remains associated with the insoluble nuclear material of immature erythroid nuclei, compared to 5% with fraction \( P_{150} \) of mature erythroid nuclei (Table 1). Thus, the proportion of the \( \beta \)-globin and histone H5 gene chromatin in the insoluble residual nuclear material is greater in immature cells, in which these genes are expressed, than in mature cells, in which these genes are potentially active (Table 1). \( \beta \)-Globin and histone H5 DNA sequences of mature and immature erythrocyes are also enriched in the 0.15 M-NaCl-soluble oligonucleosomes and polynucleosomes (Fig. 1).

In both immature and mature erythrocytes, repressed vitellogenin and competent \( \epsilon \)-globin DNA sequences are
Table 1. DNA sequence distribution among the different chromatin fractions

<table>
<thead>
<tr>
<th>Source of chromatin</th>
<th>Fraction</th>
<th>DNA (%)</th>
<th>Histone H5 (%)</th>
<th>β-Globin (%)</th>
<th>e-Globin (%)</th>
<th>Vitellogenin (%)</th>
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<tbody>
<tr>
<td>Mature chicken erythrocytes</td>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>P_F</td>
<td>4.9±0.3</td>
<td>27.4 ±3.8</td>
<td>23.0±0.5</td>
<td>6.0±2.3</td>
<td>6.6±1.3</td>
</tr>
<tr>
<td></td>
<td>S_F</td>
<td>95.1±0.3</td>
<td>72.3±8.2</td>
<td>66.7±13.4</td>
<td>88.9±14.6</td>
<td>95.3±0.0</td>
</tr>
<tr>
<td>Immature chicken erythrocytes</td>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>P_F</td>
<td>9.9±0.8</td>
<td>51.4±9.9</td>
<td>47.8±7.2</td>
<td>14.4±0.7</td>
<td>11.2±0.5</td>
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<tr>
<td></td>
<td>S_F</td>
<td>90.1±0.8</td>
<td>46.6±8.8</td>
<td>42.1±5.3</td>
<td>90.4±12.2</td>
<td>93.1±10.4</td>
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<td></td>
<td>P_F150</td>
<td>78.3±3.3</td>
<td>9.8±4.0</td>
<td>13.7±4.0</td>
<td>35.3±14.1</td>
<td>90.1±5.6</td>
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<tr>
<td></td>
<td>S_F150</td>
<td>11.8±4.1</td>
<td>38.1±9.6</td>
<td>33.5±9.2</td>
<td>50.7±8.3</td>
<td>0.8±0.4</td>
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<tr>
<td></td>
<td>F1</td>
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<td>17.6±1.8</td>
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<td></td>
<td>F11</td>
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<td>21.9±0.9</td>
<td>0.2±0.1</td>
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<tr>
<td></td>
<td>F111</td>
<td>1.6±0.3</td>
<td>8.8±1.9</td>
<td>9.2±1.3</td>
<td>5.1±0.8</td>
<td>0.2±0.0</td>
</tr>
</tbody>
</table>

not enriched in the insoluble nuclear material (P_F; Fig. 1 and Table 1). The repressed vitellogenin DNA sequences are located in the 0.15 M-NaCl-insoluble chromatin (P_F150) and 0.15 M-NaCl-soluble mononucleosomes (S_F150), but these sequences are under-represented in the 0.15 M-NaCl-soluble polynucleosomes. The competent e-globin DNA sequences are enriched in the 0.15 M-NaCl-soluble polynucleosomes of S_F150 chromatin (Fig. 1 and Table 1).

The immature erythrocyte chromatin fragments that are soluble in 0.15 M-NaCl (fraction S_F150) were fractionated by gel exclusion chromatography on a Bio-Gel A-5m column, obtaining four fractions F1, F11, F111 and F11 in of decreasing DNA lengths (see Fig. 1, DNA). Competent e-globin sequences are highly enriched in fraction F1 polynucleosomes, while active histone H5 and β-globin DNA sequences are enriched to the greatest extent in fraction F11 (Fig. 1 and Table 1). The repressed vitellogenin DNA sequences were depleted in the 0.15 mm-NaCl-soluble oligo- and poly-nucleosomes present in fractions F1, F11, and F111 (Fig. 1 and Table 1). Since the 0.15 M-NaCl-soluble oligo- and poly-nucleosomes are highly enriched in competent and active DNA sequences and depleted in repressed DNA, these chromatin fractions will be the most informative as to the composition of transcriptionally active and competent chromatin.

The 0.15 M-NaCl soluble polynucleosomes of immature erythrocytes are enriched in modified histone species

Goldknopf et al. (1980) reported that immature chicken erythrocyte chromatin had a greater amount of ubiquitinated histone H2A (uH2A) than did mature erythrocytes. We determined whether the amount of uH2B in immature erythrocyte chromatin was also elevated. Histones of immature (lane T in Fig. 2) and mature (lane Tm in Fig. 2) erythrocyte nuclei were electrophoretically resolved on an acetic acid/urea/Triton/15% polyacrylamide gel, and the amount of uH2A and uH2B was determined. As reported previously, the amount of uH2A is greater in immature than in mature erythrocyte histones (approx. 5-fold). This is also found for uH2B, with the amount of this modified histone being approx. 5-fold greater in immature erythrocyte chromatin.

The 0.15 M-NaCl-soluble polynucleosomes of mature chicken erythrocytes are enriched in acetylated species of histones H2B and H4 and ubiquitinated species of histones H2A and H2B (Ridsdale & Davie, 1987). The 0.15 M-NaCl-soluble immature erythrocyte oligo- and poly-nucleosomes are also enriched in these modified histone species (see lanes F1, F11, and F111 in Fig. 2). When the amount of the ubiquitinated histones of the 0.15 M-NaCl-soluble oligo- and poly-nucleosomes (lanes F1, F11 and F111) was compared to that of unfractionated chromatin (lane T), uH2A and uH2B were enriched approx. 1.7-fold and 3.2-fold, respectively. This enrichment is comparable to that observed for the 0.15 M-NaCl-soluble polynucleosomes of mature erythrocytes.
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Fig. 3. Identification of ubiquitinated histone species in the different immature erythroid chromatin fractions

Acid-soluble proteins (9 μg) isolated from each immature erythroid fraction were electrophoretically separated on two-dimensional gels (AUT into SDS). The proteins were electrophoretically transferred to nitrocellulose and immunochromatically stained for ubiquitin with anti-ubiquitin IgG and 125I-labelled protein A. The autoradiograms are shown. The polyubiquitinated histone species are labelled as u₂, u₃ and u₄, representing the attachment of two, three and four ubiquitins respectively.

(Ridsdale & Davie, 1987). However, it should be noted that the amount of ubiquitinated histones of the 0.15 M-NaCl-soluble polyribosomes of immature erythrocytes is approx. 5-fold greater than the amount of uH2A and uH2B of 0.15 M-NaCl-soluble polyribosomes of mature cells. None of the other chromatin fractions, including F₅, are enriched in acetylated histone species or uH2A. However, uH2B appears to be enriched in fraction F₇. The histones of immature erythrocyte chromatin fractions F₅ and T were electrophoretically resolved on SDS/polyacrylamide gels (not shown). Analysis of the amount of linker histones H1 and H5 in the histones of these two chromatin fractions shows that fraction F₅ has approx. 50% of the amount of linker histones found in unfractionated chromatin.

The distribution of the ubiquitinated and polyubiquitinated histone species among the immature erythroid chromatin fractions was determined. Similar amounts of histones from the various fractions were resolved by two-dimensional gel electrophoresis, transferred to nitrocellulose, and the ubiquitin-protein conjugates were detected by immunochromenochemical staining with an anti-ubiquitin IgG and 125I-protein A. Fig. 3 shows that uH2B and the polyubiquitinated species of H2A are enriched in the 0.15 M-NaCl-soluble oligo- and poly-nucleosomes of fractions F₁, F₂ and F₃. The histones of fraction F₅ are not enriched in uH2B, but histone H2A is enriched in polyubiquitinated species.

A range of nuclease digestions does not alter the enrichment of active DNA in the low-salt-insoluble nuclear material

Rose & Garrard (1984) observed that increasing the fragmentation of MPC-11 chromatin resulted in the release of the active κ immunoglobulin light chain gene sequences from the low-salt-insoluble nuclear material into a salt-soluble chromatin fraction. To test whether increasing the extent of nuclease digestion would result in liberation of the active DNA sequences from the low-salt-insoluble nuclear material into fraction S₁₅₀, mature and immature chicken erythrocyte nuclei were incubated for various times with micrococcal nuclease, and the chromatin was fractionated. Fig. 4 shows the distribution of the histone H5 gene sequences in chromatin fractions F₁ and S₁₅₀. The results demonstrate that increasing the extent of nuclease digestion reduces the average size of the histone H5 DNA fragments, but not the magnitude of enrichment in the insoluble chromatin fraction F₁. Moreover, increasing the digestion time to 60 min had little effect on the selective partitioning of the active histone H5 gene sequences with the insoluble nuclear material (not shown).

Among the smear of histone H5 DNA fragments, two discrete bands of about 2.3 kb and 1.15 kb are visible for each fraction isolated from the mature erythrocyte nuclei. These bands are probably generated by digestion at the micrococcal hypersensitive sites (MHS) which are located at positions −111 ± 6 (MHS 5), 1016 ± 12/1211 ± 12 (MHS 7) and 2182 ± 30 (MHS 8) (Renaud & Ruiz-Carrillo, 1986). Preferential digestion at MHS 5 and MHS 8 would generate a band of approx. 2.3 kb, while preferential digestion at MHS 5 and 7 or MHS 7 and 8 would generate a band of approx. 1.15 kb. As the extent of digestion increases, the intensity of the 2.3 kb fragment decreases while that of the 1.15 kb fragment increases. In immature erythrocytes, the 2.3 kb band is less distinct in all fractions and is absent from the S₁₅₀ fractions, where the majority of the H5 DNA fragments are shorter than...
Immature or mature erythocyte nuclei were incubated for 10, 20 and 30 min with micrococcal nuclease. DNA (8 µg) isolated from nuclei (T), the insoluble nuclear $P_R$ fractions (P) or the 0.15 M-NaCl-soluble $S_{150}$ fractions (S) was run on 1 % agarose gels, stained with ethidium bromide (DNA) or transferred onto nitrocellulose and hybridized to the $^{32}$P-labelled histone H5 probe.

1.15 kb. The 1.15 kb band is quite apparent in the 10 min digestion $S_{150}$ fraction. Note that, in contrast to mature erythroid chromatin, reducing the extent of fragmentation of immature erythroid chromatin does not result in the solubilization of long histone H5 gene chromatin fragments into fraction $S_{150}$.

The loss of RNA or acetylated histone species does not alter the enrichment of active genes with the low-salt-insoluble nuclear material of immature erythrocytes

The nature of the association of the active genes with the insoluble nuclear material in fraction $P_R$ of immature erythrocytes was investigated. Fraction $P_R$, was incubated with RNAase, centrifuged and DNA was isolated from the treated insoluble nuclear material. Fig. 1, lane $P_R$, shows that RNAase digestion does not alter the enrichment of active DNA sequences in this fraction, suggesting that the active gene sequences are not attached to the residual nuclear material via RNA.

Alonso et al. (1987) demonstrated that the removal of acetyl groups from the hyperacetylated histones resulted in a loss of the low-salt solubility of the β-globin and histone H5 gene chromatin. Conversely, the amount of low-salt-soluble active gene chromatin was increased by augmentation of the amount of hyperacetylated histones (D. A. Nelson et al., 1986). This was achieved by incubating cells in the presence of butyrate, an inhibitor of histone deacetylase (Candido et al., 1978). To determine whether changing the amount of hyperacetylated histones would alter the partitioning of the active genes with the low-salt-insoluble nuclear material, immature erythroid cells were incubated in the presence or absence of sodium butyrate under conditions known to hyperacetylate or hypoacetylate histones of active chromatin (Alonso et al., 1987). The enrichment of β-globin and histone H5 DNA sequences in fraction $P_R$ was not altered (not shown).

The enrichment of active DNA with the low-salt-insoluble nuclear material is not dependent on on-going transcription

Immature erythrocytes were incubated with or without transcription inhibitors, actinomycin D and DRB. The presence of actinomycin D and DRB or DRB alone did not affect the distribution of active histone H5 sequences among the different fractions, and the enrichment in the insoluble nuclear fraction $P_R$ was not altered (not shown).

DISCUSSION

We demonstrate that the erythroid-specific β-globin and histone H5 genes are enriched in the low-salt-insoluble nuclear material of immature and mature chicken erythrocytes. Approx. 50 % of the active β-globin and histone H5 gene chromatin of immature erythrocytes and approx. 25 % of the chromatin of these potentially active genes of mature erythrocytes have an insoluble nature. The histone H2A.F gene (also known as H2A.Z), which is expressed in immature erythrocytes (Harvey et al., 1983), has the same distribution among the various chromatin fractions of immature or mature erythrocytes as that of the β-globin and histone H5 genes (not shown). In contrast, the competent ε-globin gene, which is part of the DNAase I-sensitive β-globin domain and is located next to the β-globin gene, is not enriched in the DNA of the residual nuclear material. In addition, genes, such as thymidine kinase and c-myc, that are no longer expressed in immature erythrocytes (Conklin & Groudine, 1986) and repressed genes (vitellogenin and ovalbumin) are not enriched in the low-salt-insoluble nuclear material. These results suggest that only those genes that are expressed or have the potential for expression (for example, the β-globin gene of mature erythrocytes that have RNA polymerase molecules
located at the 5' end of the gene) are enriched in the low-salt-insoluble nuclear material of immature or mature erythrocytes. Furthermore, gene sequences that are sensitive to DNAase I (e.g. e-globin) are not necessarily enriched in the insoluble nuclear material.

Since immature and mature erythrocytes lack an internal nuclear matrix (Lafond & Woodcock, 1983; Lafond et al., 1983), this suggests that the insolubility of active genes is not due to their association with the internal matrix. Several reports indicate that RNA is essential for the maintenance of the internal matrix structure (for review see Verheijen et al., 1988; Nickerson et al., 1989). Incubation of the residual nuclear material with RNAase did not solubilize active gene chromatin. This supports the conclusion that the insolubility of active gene chromatin is not a consequence of an interaction of active genes with the internal matrix. Moreover, our results suggest that the insolubility of active gene sequences is not due to the formation of intermolecular disulphide bonds, amount of histone acetylation or presence of RNA polymerase. Kaufmann et al. (1986) reported that disulphide bonds may mediate the association between the glucocorticoid receptor and nuclear matrix. It is conceivable that the presence of proteins that are capable of forming disulphide bonds with active genes may mediate the attachment of active genes with the nuclear matrix. However, the addition of iodoacetamide to the buffer used to isolate the chicken erythrocyte nuclei would prevent the formation of such bonds. The incubation of immature erythrocytes in the presence of DRB, which blocks transcription at or near the initiation site (Andersson et al., 1982; Zandomeni et al., 1986), does not alter the enrichment of active histone H5 DNA in fraction $P_{c}$. DRB does not intercalate or interfere with elongation of transcription and allows the release of RNA polymerase upon termination of transcription (Zandomeni et al., 1983). This suggests that the association of active genes with the insoluble nuclear pellet of immature erythrocytes is not due to the presence of RNA polymerase. Our results are consistent with the idea that the insolubility of active gene chromatin is a result of the presence of preinitiation transcription complexes. Culotta et al. (1985) observed the quantitative sedimentation of stable RNA polymerase II preinitiation complexes formed by incubation of a naked DNA template and a whole-cell extract. These complexes lacked a factor necessary for transcription, so the insolvability was independent of transcription.

In mature and immature cells, the majority of the EDTA-solubilized active/potentially active $\beta$-globin and histone H5 gene chromatin fragments are soluble in 0.15 M-NaCl (the present paper; Ridsdale & Davie, 1987; Ridsdale et al., 1988). The majority of the salt-soluble immature erythrocyte histone H5 gene chromatin fragments are of lengths 1.15 kb or shorter, while the 0.15 M-NaCl-soluble histone H5 gene chromatin fragments of mature erythrocytes can be considerably longer. It is interesting to note that 1.15 kb is the distance between micrococal-nuclease-hypersensitive sites (MHS) 5 and 7, which are located at the 5' and 3' ends of the histone H5 gene respectively (Renaud & Ruiz-Carrillo, 1986). It is conceivable that only the coding region of the active histone H5 gene chromatin of immature cells is salt-soluble, while the coding and flanking regions of the potentially active histone H5 gene chromatin of mature cells are soluble in 0.15 M-NaCl.

The protein composition of the 0.15 M-NaCl-soluble oligo- and poly-nucleosomes, which are depleted in repressed DNA and enriched in active/competent DNA, of immature erythroid cells is similar to that of mature erythrocytes. However, the 0.15 M-NaCl-soluble poly-nucleosomes of immature erythrocytes have increased levels of H2A and H2B. It is possible that the increased amounts of the ubiquitinated histones may generate active/competent chromatin domains in immature erythrocytes that have a more 'open', disrupted, structure than the potentially active/competent chromatin domains of mature erythrocytes. The 0.15 M-NaCl-soluble poly-nucleosomes from both sources are enriched in hyperacetylated species of histones H4 and H2B. Recently, Hebbes et al. (1989) reported that acetylated histone species of the 0.15 M-NaCl-soluble oligonucleosomes are complexed with active genes. Interestingly, histone acetyltransferases are enriched in the linker DNA regions of the 0.15 M-NaCl soluble poly-nucleosomes (Chan et al., 1988). It will be of considerable interest to determine whether other enzymes involved in modifying the histones are also preferentially localized with the active/competent gene chromatin domains.

Polyubiquitinated species of H2A are enriched in the 0.15 M-NaCl-soluble oligo- and poly-nucleosomes and in the insoluble chromatin fraction $P_{c}$. Recently, we determined that the major arrangement of ubiquitin in polyubiquitinated H2A is a chain of ubiquitin molecules joined to each other by isopeptide bonds to a ubiquitin molecule which is attached to the e-amino group of Lys-119 of histone H2A (Nickel & Davie, 1989b). The role of histone polyubiquitination is not known. It is possible that polyubiquitination of the histones alters nucleosome structure and/or nucleosome packaging into higher order chromatin structures. Another possibility is that polyubiquitinated histones are tagged for turnover when displaced from the DNA. It is interesting to note that Chau et al. (1988) observed that cytoplasmic proteins tagged with a chain of ubiquitin molecules, but not those that were mono-ubiquitinated, were recognized in ubiquitin-dependent degradation of proteins.

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