Volume 331, number 1,2, 141–144 FEBS 13025 September 1993


# Repression of histone H5 gene expression in chicken mature erythrocytes is correlated with reduced DNA-binding activities of transcription factors SP1 and GATA-1

Jian-Min Sun, C. Gail Penner, James R. Davie*

*Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Man., R3E 0W3, Canada*

Received 9 August 1993

During the final stages of erythroid maturation, the expression of the chicken histone H5 gene ceases. The histone H5 promoter has binding sites for Sp1 and UPE-binding protein. The 3′ histone H5 enhancer has binding sites for Sp1, GATA-1 and NF1. Here, we show that the DNA-binding activities of transcription factors Sp1 and GATA-1 is reduced 5- to 10-fold in mature cells, while the activities of UPE-binding protein and NF1 remain the same in mature and immature erythrocytes. The reduced activities of Sp1 and GATA-1 may contribute to the inactivation of the histone H5 gene in mature erythrocytes.

Transcription; Transcription factor; Gene expression; Histone H5 gene

## 1. INTRODUCTION

Chicken histone H5 is a H1-like linker histone that is expressed only in nucleated erythrocytes [1]. Levels of histone H5 protein increase during the differentiation and maturation of the chicken erythroid cell, and this increase is correlated with chromatin condensation, the shut down of replication and the repression of gene expression [2]. Erythroid-specific $\beta$-globin and histone H5 genes, for example, are transcriptionally active in adult chicken immature erythrocytes but not in mature cells [3].

Using adult chicken immature erythrocyte nuclear extracts, we demonstrated that the histone H5 gene promoter has binding sites for Sp1 (high affinity) and an upstream promoter element (UPE)-binding protein, while the 3′ enhancer contains binding sites for Sp1 (one of medium- and three of weak affinity, one of which may also bind the CACCC factor), GATA-1 and NFI [4]. Both Sp1 and GATA-1 bind to their respective DNA binding sites as multimeric complexes [4–6]. We proposed that Sp1 multimers bound at the promoter and enhancer interact to mediate the juxtapositioning of the enhancer and promoter elements, bringing the GATA-1 multisubunit complex next to the initiation site [4]. In this study, we show that the amounts of Sp1 and GATA-1 complexes (as measured by their ability to bind to their specific DNA-binding sites in a gel mobility-shift assay), but not NF1 and UPF-binding protein, are considerably lower in mature erythrocyte nuclei than in immature erythroid nuclei. These results suggest that the repression of the histone H5 gene and other erythroid-specific genes is partly a consequence of transcription factor abundance.

*Corresponding author. Fax: (1) (204) 783-0864.

## 2. MATERIALS AND METHODS

### 2.1. *Preparation of nuclear extracts*

Adult White Leghorn chickens were made anaemic by injections of 1-acetyl-2-phenylhydrazine Blood was collected, and nuclei were isolated from immature and mature erythrocytes as described previously [4]. Nuclei were extracted with RSB (10 mM Tris-HCl, pH 7.5, 10 mM NaCl, 3 mM $MgCl_2$, 10 mM sodium butyrate, 1 mM phenylmethanesulphonyl fluoride, 1 $\mu$M leupeptin, 0.15 $\mu$M aprotinin, 5 $\mu$M iodoacetamide) containing 0.3 M NaCl [4].

### 2.2. *DNA fragments and oligonucleotides*

DNA fragments spanning the histone H5 5′ promoter and 3′ enhancer of H5 gene (see Fig. 1) were isolated as described previously [4]. Oligonucleotides containing the histone H5 GC-box (Sp1 oligonucleotide), which has the sequence of a high-affinity Sp1 binding site in the promoter region, upstream promoter element (UPE oligonucleotide), and GATA site (GATA oligonucleotide) were synthesized as described [4]. Oligonucleotides that contained the NF1 motif (NF1 oligonucleotide), 5′-TCGAGGGCTTGGCACAGCCCCAAGACC-A-3′ (NF1-for) and 5′-GTGGTCTTGGGGCTGTGCCAAGCCCT-CG-3′ (NF1-rev), were synthesized.

### 2.3. *DNA-binding assays*

DNA end-labelling, gel mobility-shift (EMSA), competition experiments, and DNase I protection (footprinting) assays were done as described previously [4,7].

## 3. RESULTS

The adult chicken immature erythroid nuclear factors

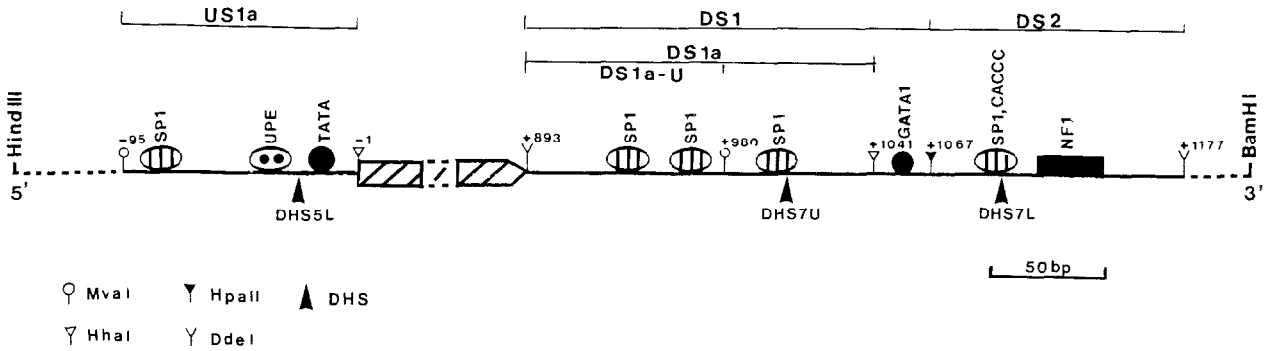


Fig. 1. Map of the chicken histone H5 gene and flanking regions is shown. The proteins of adult chicken immature erythrocyte nuclear extracts that bind to the sequence-specific domains are indicated. The DNA fragments used in this study (US1a, DS1, DS2, DS1a and DS1a-U) are shown. The arrowheads show the location of the DNase I hypersensitive sites (DHS) in the erythroid histone H5 gene chromatin. The transcription factor binding sites are symbolized.

that bind to the histone H5 promoter and enhancer regions in vitro are shown in Fig. 1. The promoter region has a high-affinity Sp1-binding site and a site that binds to the UPE-binding protein. The downstream enhancer has binding sites for GATA-1, NF1 and Sp1. The Sp1 binding sites were of medium and weak affinity [4].

The electrophoretic gel mobility-shift assay (EMSA) with DNA fragments US1a, DS1 and DS1a-U was used to detect chicken immature and mature erythroid nuclear proteins that interacted with the histone H5 promoter and enhancer regions. The major complexes formed with US1a and immature erythrocyte nuclear extracts were C7 and C8 (Fig. 2) [4]. Previously, we demonstrated that these complexes were effectively competed for by an oligonucleotide containing a high-affinity Sp1-binding site (Sp1 oligonucleotide) [4], providing evidence that Sp1 was involved in the formation of these complexes. Further, we presented evidence that these high molecular weight complexes contain multimers of Sp1 which have been described by others [5,6]. The abundance of the complexes C7 and C8 was low in mature erythrocyte nuclear extracts. We observed that the amounts of these complexes in different mature erythrocyte nuclear extracts was variable, but their levels were always considerably lower than those of immature erythrocyte nuclear extracts. Furthermore, the complexes C4 and C5 that formed with DS1a-U were lower in abundance in mature erythrocyte nuclear extracts than in immature erythrocyte nuclear extracts. These complexes were also shown to be effectively competed for by the Sp1 oligonucleotide [4].

These observations suggested that mature erythrocyte nuclear extracts had low levels of Sp1. This was confirmed using EMSA with Sp1 oligonucleotide and mature or immature erythrocyte nuclear extracts. Fig. 2B shows that Sp1-binding activity was reduced in mature erythrocyte nuclear extracts which had approximately 10–20% of the Sp1 activity present in immature erythrocyte nuclear extracts.

The major complex formed with mature nuclear extracts and the US1a fragment was C6. Previously, we had shown that a UPE oligonucleotide effectively competed for complex C6 [4]. EMSA with a UPE oligonucleotide, and nuclear extracts from immature and mature erythrocyte nuclear extracts, demonstrated that the DNA-binding activity of the UPE-binding proteins was similar in both cell types (Fig. 2B).

The relative levels of complexes C1, C2, C3 and C4 formed with DS1 were lower in mature erythrocyte nuclear extracts than in immature erythrocyte nuclear extracts. These complexes were generated by the association of GATA-1 (C1) or GATA-1 complexes (C2, C3 and C4) with the GATA binding site of DS1 [7]. EMSA with GATA oligonucleotide and either mature or immature erythrocyte nuclear extracts demonstrated that the levels of the GATA-1 and GATA-1 multisubunit complexes were low in mature erythrocyte nuclear extracts (approximately 10% of the immature erythrocyte nuclear GATA-1 activity) (Fig. 2B).

In contrast to GATA-1 and Sp1, the DNA-binding activity of NF1 was similar in nuclear extracts from mature and immature erythrocytes (Fig. 2B). Both nuclear extracts formed a similar spectrum of complexes with a NF1 oligonucleotide that contained the NF1-binding site in the histone H5 enhancer. These complexes were effectively competed for with unlabelled NF1 oligonucleotide but not with the Sp1 oligonucleotide (not shown).

Fig. 3 shows DNase I footprinting analysis of DNA fragments spanning either the histone H5 promoter or enhancer and nuclear extracts of immature and mature erythrocytes. The high-affinity Sp1 binding site of the promoter was protected with either immature or mature erythrocyte nuclear extracted proteins. With different mature erythrocyte nuclear extracts, this high-affinity Sp1 binding site was generally well protected. However, the weaker Sp1-binding sites in the enhancer region were less protected with nuclear extracts from mature cells than with those from immature erythrocytes. The

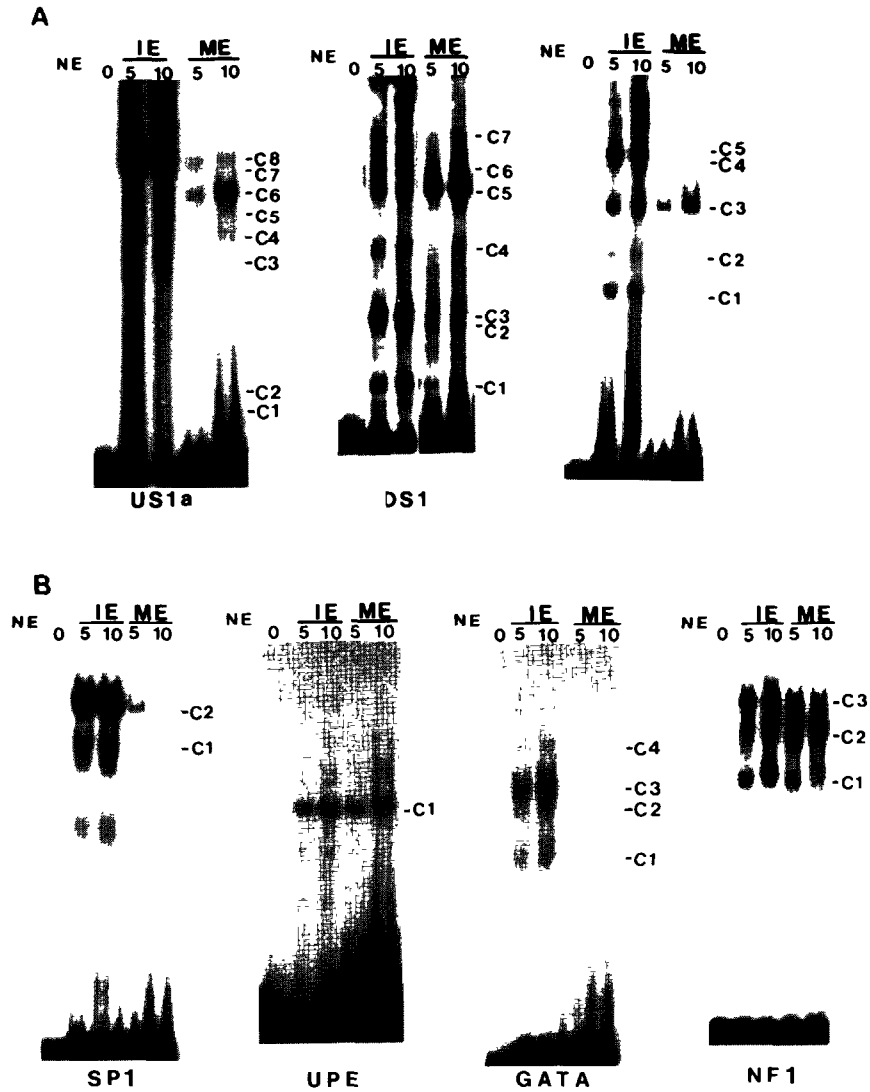


Fig. 2. Transcription factor levels of adult chicken immature and mature erythrocytes. The DNA fragments or oligonucleotides used in the electrophoretic gel mobility-shift assays are indicated at the bottom of each panel. (A) Histone H5 upstream DNA fragment, US1a, and downstream DNA fragments, DS1, DS1a-U, were incubated with nuclear extracts (NE) isolated from immature (IE) or mature (ME) erythrocytes 1 ng of end-labelled DNA fragments was incubated with either 5 or 10 $\mu$g of protein. (B) end-labelled oligonucleotides were incubated with 5 or 10 $\mu$g of immature or mature erythrocyte nuclear extracted protein. C1–C8 are the protein–DNA complexes.

degree of protection at the Sp1-binding sites in the enhancer region varied with the different mature erythrocyte nuclear extracts, reflecting the different levels of Sp1 activity in these preparations. The level of protection at these enhancer Sp1-binding sites generated with mature erythrocyte nuclear extracts was always less than that protected by immature erythrocyte nuclear extracts. Similarly, the protection of the GATA-binding site was always greater with immature erythrocyte nuclear extracts than with mature erythrocyte nuclear extracts. In contrast, the UPE- and NF1-binding sites were similarly protected with nuclear extracts from immature and mature erythroid nuclear extracts.

## 4. DISCUSSION

It had been previously assumed that the levels of Sp1 are at similar levels throughout all stages of erythroid development [8]. However, a more recent report shows that the concentrations of Sp1 and GATA-1 decline throughout development as the primitive erythroid cells are replaced by the definitive lineage [9]. Our results demonstrate that the abundance of the transcription factors, Sp1 and GATA-1, decrease further in the terminally deferentiated mature erythrocyte which ceases to express the histone H5 and $\beta$-globin genes. Other factors such as UPE-binding protein and NF1 are at similar amounts in mature and immature erythrocytes. It should be noted that the gel mobility-shift assay (EMSA) was used in these studies to measure the nu-

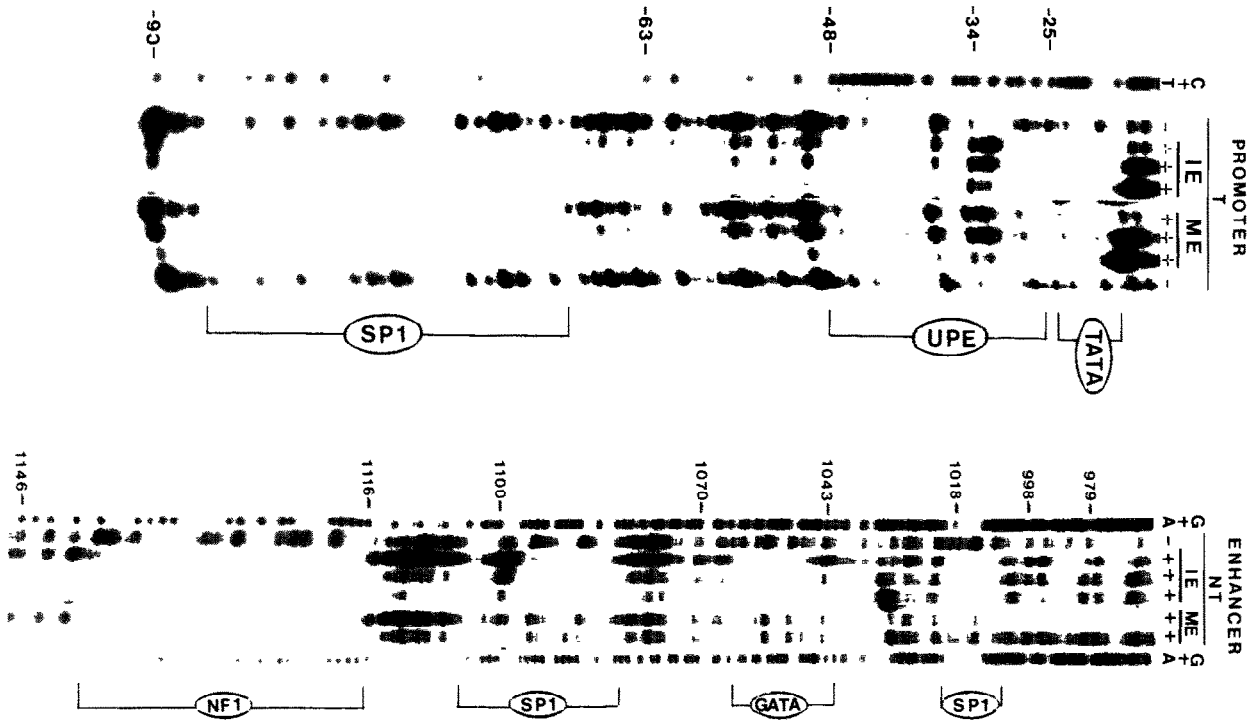


Fig. 3. DNase I footprint analysis of adult chicken immature and mature erythrocyte nuclear extract proteins interacting with the histone H5 promoter and enhancer regions. DNA fragments (1–2 ng) US1a (promoter) or DS1 + DS2 (enhancer) were end-labelled on the template (T) or non-template (NT) strand, and incubated with (+) or without (−) 10–30 μg nuclear extracts isolated from immature (IE) or mature (ME) erythrocytes. C+T or G+A chemical sequencing reactions are indicated. The amount of DNase I added to 50 μl was as follows (left to right): 0.2, 0.1, and 0.01 units. For enhancer panel (ME) the units of DNase I were 0.2 and 0.1 units. The factor binding sites are indicated.

clear concentration of factors that are capable of binding to specific DNA sequences. Modified factors that cannot function in either DNA binding or transactivation will not be detected by this assay [9].

Transcription factors have been shown to leak out of the erythrocyte nuclei during their isolation [8,10]. However, when nuclear extracts and cytoplasmic supernatants from mature erythrocytes were analyzed by EMSA, we observed that the majority of the Sp1 and GATA-1 activities were present in the nuclear extracts (not shown).

We proposed a model in which Sp1 multimers bound at the promoter and enhancer of the histone H5 gene interact to mediate the juxtapositioning of the enhancer and promoter elements [4]. This would bring the GATA-1 multisubunit complex next to the initiation site. The reduction in Sp1 and GATA-1 multisubunit complexes in mature erythrocytes would seriously compromise the positioning and function of the histone H5 enhancer.

Chicken mature erythrocytes contain the factor, cIBR, which binds to the transcription start site of the histone H5 gene and represses its expression [3]. cIBR is not present in early erythroid cells that express the histone H5 gene [3]. Further, the inactivation of the general transcription factor, TFIIA, may also contribute to the inactivation of the histone H5 in mature erythrocytes [11]. Together, these observations suggest that several mechanisms are involved in the specific repression of the histone H5 gene.

*Acknowledgements.* This project was supported by a grant from the Medical Research Council of Canada (MT-12147) and by a Medical Research Council Scientist award to J.R.D

## REFERENCES

[1] Neelin, J M., Callahan, P.X., Lamb, D.C and Murray, K. (1964) Can. J. Biochem. 42, 1743–1752.
[2] Sun, J.M., Wiaderkiewicz, R. and Ruiz Carrillo, A. (1989) Science 245, 68–71.
[3] Gomez-Cuadrado, A., Rousseau, S., Renaud, J. and Ruiz Carrillo, A. (1992) EMBO J. 11, 1857–1866.
[4] Sun, J.-M., Penner, C.G. and Davie, J.R. (1992) Nucleic Acids Res. 20, 6385–6392.
[5] Mastrangelo, I.A., Courey, A.J., Wall, J.S., Jackson, S.P. and Hough, P.V. (1991) Proc. Natl. Acad. Sci. USA 88, 5670–5674.
[6] Pascal, E. and Tjian, R (1991) Genes Dev. 5, 1646–1656.
[7] Penner, C.G. and Davie, J.R. (1992) Biochem. J. 283, 905–911.
[8] Jackson, P.D., Evans, T., Nickol, J.M. and Felsenfeld, G. (1989) Genes Dev. 3, 1860–1873.
[9] Minie, E.M., Kimura, T. and Felsenfeld, G. (1992) Development 115, 1149–1164.
[10] Emerson, B.M., Nickol, J.M., Jackson, P.D. and Felsenfeld, G (1987) Proc Natl. Acad. Sci. USA 84, 4786–4790.
[11] Bungert, J., Waldschmidt, R., Kober, I. and Seifart, K.H. (1992) Proc. Natl Acad. Sci. USA 89, 11678–11682.