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Transcription factor GATA-1-multiprotein complexes and chicken erythroid development

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Abstract

The chicken erythrocyte transcription factor, GATA-1, is associated with several non-DNA binding proteins. We show that GATA-1 multiprotein complexes exist in primitive and definitive erythrocytes. These complexes bind to GATA motifs of the ρ-globin promoter and histone H5 enhancer with high affinity, and to the chicken β-globin promoter specialized TATA element and enhancer GATA with low affinity. The low affinity β-globin TATA element would allow basal transcription factors to displace the GATA-1 multiprotein complex. Further, our results suggest that ρ-globin promoter's low affinity Sp1 binding site and reduced levels of Sp1 in definitive cells prevent its expression in these cells.

Key words: GATA-1; Transcription factor; Hemoglobin switching; Chicken erythropoiesis

1. Introduction

During chicken embryogenesis there is a developmentally regulated switch in the expression of the β-globin genes. The first hemoglobinized cells appear in the blood islands in chicken embryos at 35 h [1,2], and these primitive cells produce embryonic β-globin genes ρ and ε [3]. Between the 5th and 12th day, this primitive group of erythroid cells is replaced by definitive red blood cells which synthesize adult β-globins H and A [4]. The transcription of the latter two genes in red blood cells arising from the bone marrow is maximal at about days 9–14 after fertilization. This changing pattern of globin isotypes during development is called hemoglobin switching.

GATA-1 is a transcription factor that regulates most, if not all, erythroid-specific genes. This sequence-specific DNA-binding protein, which is expressed at all stages of erythropoiesis, binds to the GATA motif (WGATAR: W = A or T; R = A or G) found in the promoters and enhancers of erythroid-specific genes. The level of GATA-1 changes throughout chicken erythroid development. GATA-1 activity is higher in primitive erythroid stages than in definitive cells [5]. The decline in GATA-1 activity is accompanied by decreases in the activity of the transcription factor, Sp1. These alterations in the activities of GATA-1 and Sp1 are thought to be involved in the cessation of expression of the embryonic β-globin genes in definitive erythrocytes [5].

We have shown that GATA-1 binds to its target sequence as a multiprotein complex [6]. In this report we analyzed nuclear extracts from erythrocytes of chick embryos at various stages of development to find whether proteins that bind to GATA-1 exist in primitive erythrocytes, at a time when the embryonic β-globin genes are expressed. Further, we investigated the relative affinities of the GATA-1 multiprotein complexes for oligonucleotides with the GATA motif found in the promoters and enhancers of several erythroid specific genes, including histone H5, ρ-globin and β-globins genes. Our results show that GATA-1 binding proteins are present in both primitive and definitive erythrocytes. Further, we found that the GATA motif of the histone H5 enhancer and ρ-globin promoter, but not the β-globin enhancer, are high-affinity binding sites for GATA-1 multiprotein complexes.

2. Materials and methods

2.1. Collection of erythrocytes from chicken embryos and preparation of nuclear extracts

Chicken embryos were staged according to Hamburger and Hamilton [7]. Erythrocyte nuclei were isolated from chicken embryo erythrocytes or adult chicken immature erythrocytes, and nuclear extracts were prepared as described previously [5,8].

2.2. Oligonucleotides

Several sequences containing GATA-binding sites of regulatory elements in erythroid-specific genes were synthesized. The H5 enh oligonucleotide sequence covers the region from + 1,045 to + 1,067

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downstream of the start of transcription of the chicken H5 gene, and covers the GATA-protein binding site [8,9]. The complementary sequences of this site are GAGGCTGGAGATAACAGTGCGG (H5 enh-for) and GCCGCACTGTTATCTCCAGCCT (H5 enh-rev). The βG enh oligonucleotide contains the GATA sequence of the chicken β-globin enhancer located from + 1,888 to + 1,910 downstream of the start of transcription [10]. The synthesized sequences are CAGGTTGCAGATAAACATTTTG (βG enh-for) and GCAAAATGTTTATCTGCAACCT (βG enh-rev). The oligonucleotides encoding the chicken β-globin promoter sequence, which has a GATA sequence in place of the TATA box, were GGCGGAGGCGATAAAAGTGGGG (TATA/ GATA-for) and TCCCCACTTTTATCGCCTCCGC (TATA/GATA- rev) [11]. The chicken ρ-globin promoter sequence GGACAGCAAGATAAGGGCTGCT (ρ promoter-for) and CAGCAGCCCTTATCTTGCTGTC (ρ promoter-rev) were synthesized (−200 to −206) [5]. The mouse α-globin promoter sequences (located at −189 to −174) GGGGCAACTGATAAGGATTCCC (αG2 promoter-for) and TGGGAATCCTTATCAGTTGCCC (αG2 promoter-rev) were synthesized [12].

2.3. DNA-binding assays

DNA end-labelling, gel mobility-shift (EMSA), and competition experiments were done as described previously [6,8].

4. Results

3.1. Identification of DNA-binding proteins in GATA-1 multiprotein complexes

We have shown that nuclear extracts from adult chicken immature erythrocytes form several complexes

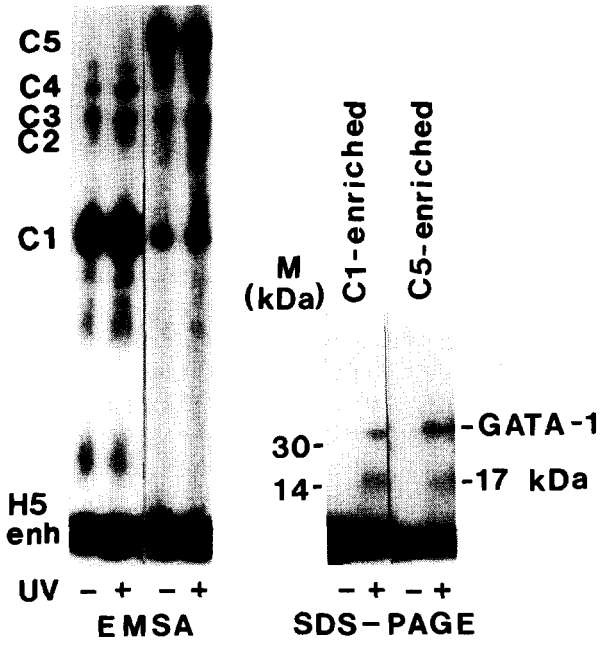

Fig. 1. Detection of DNA-binding proteins in protein–DNA complexes forming with the histone H5 enhancer GATA sequence. The uniformly labelled H5 enh oligonucleotide (0.5 ng) was incubated with adult chicken immature erythrocyte nuclear extracted protein (50 μg) enriched in C1 or C5 activity. The incubation mixtures were (+) and were not (−) irradiated with ultraviolet light and analyzed by EMSA (left panel). H5 enh is the free DNA and C1–C5 are the protein–DNA complexes. The protein–DNA complexes were digested with DNase I and micrococcal nuclease, and the affinity-labelled proteins were analyzed on a 12% polyacrylamide SDS gel. The positions of the molecular mass markers (M) are shown.

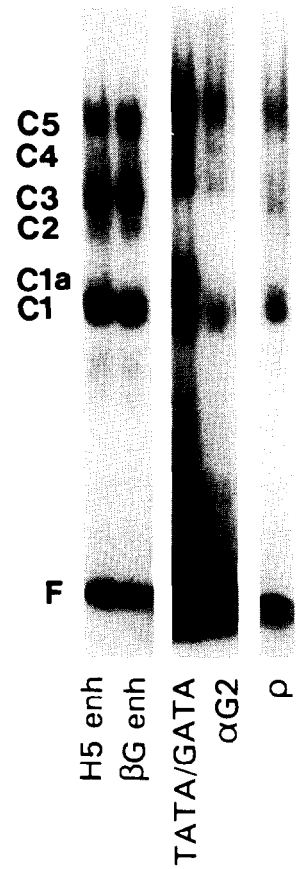

Fig. 2. Interaction of GATA-1 and GATA-1 multiprotein complexes with GATA-binding sites found in regulatory elements of erythroid-specific genes. EMSAs were done with oligonucleotides (0.125–0.5 ng) with variant GATA sequences (see Table 1), which were labelled to different specific activities, and nuclear extracted proteins (10–18 μg) isolated from adult chicken immature erythrocytes. C1–C5 are the protein–DNA complexes, and F is the free oligonucleotide.

(C1–C5) with an oligonucleotide containing the GATA motif of the histone H5 3′ enhancer [6,8]. Our results with the electrophoretic mobility shift assay (EMSA) suggested that GATA-1 generated complex C1, while proteins interacting with GATA-1 generated complexes C2–C5 (see Fig. 1) [6]. Further evidence that GATA-1 is the sole DNA-binding protein of these complexes is shown in Fig. 1. By exploiting the observation that the GATA-1 multiprotein complexes readily dissociated under certain dialysis conditions (e.g. dialysis against HEPES-containing buffer D) [8], we varied the relative abundances of the complexes generated by the nuclear extracts from mainly C5 to predominantly C1. These nuclear extracts from adult chicken immature erythrocytes were incubated with radiolabelled H5 enh oligonucleotide and irradiated with UV light. Proteins of 37 (GATA-1) and 17 kDa were cross-linked by ultraviolet light with each of the nuclear extract preparations. In four repetitions of this experiment, the DNA-binding protein in preparations enriched in either complex C1 (GATA-1 only) or complexes C2–C5 had a molecular mass of 37–40 kDa. This

included nuclear preparations in which the major complex was C3 or C5. This indicates that GATA-1 is the only DNA binding protein of the complexes, C2–C5.

3.2. DNA-binding specificity of GATA-1 multiprotein complexes

Several naturally existing GATA-1 binding sequences that are found in the promoter or enhancer elements of erythroid-specific genes were synthesized and used in EMSA (Table 1). Fig. 2 shows the results of the EMSAs with these oligonucleotides and nuclear extracts from adult chicken immature erythrocytes. GATA-1 (C1) and GATA-1 multiprotein complexes (C2–C5) formed with each oligonucleotide. Besides generating GATA-1 and GATA-1 multiprotein complexes, the β-globin TATA/GATA sequence also formed a complex of slower mobility than complex C1 (see complex C1a in Fig. 2). Basal transcription factors also bind to this specialized TATA box [11,13]. It is possible that complex C1a was produced by the binding of basal transcription factors (e.g. TATA-binding protein and TFIIA) [13].

Competition experiments were done to estimate the relative affinities of the GATA-1 multiprotein complexes for variant GATA motifs. The H5 enh oligonucleotide was radiolabelled and competed with either self or the other GATA variant oligonucleotides at 2.5 molar excess over the labelled H5 enh oligonucleotide. The ρ promoter and αG2 promoter oligonucleotides were also used in competitions at 1.25 and 0.625 molar excesses. Fig. 3 shows that each of the competitor oligonucleotides decreased the binding of GATA-1 (C1) and GATA-1 multiprotein (C2–C5) complexes to the radiolabelled H5 enh oligonucleotide. Further, the reduction in binding of the GATA-1 multiprotein complexes was similar to that of GATA-1 alone (complex C1). The strength of competition was αG2 promoter ≥ ρ promoter (1.2) ≥ H5 enh (1.3) > TATA/GATA (2.8) > βG enh (3.1). (The approximate molar excess of competitor oligonucleotide required to attain a similar amount of competition for the formation of complex C1 or C5 as the αG2 promoter oligonucleotide at one molar excess is given).

3.3. GATA-1 multiprotein complexes in primitive and definitive red blood cell nuclei

Chicken erythrocytes of stage 28 (days 5–6, primitive), stage 38 (day 12, definitive) and stage 40 (day 14, definitive) embryos were isolated. To avoid dissociation of the GATA-1 multiprotein complexes, nuclear extracts of the red blood cells were prepared without dialysis. When these fractions were used in EMSAs, the pattern of GATA-1-containing complexes was similar to that formed by extracts of adult immature erythrocytes. GATA-1 complexes C1–C5 were formed by each extract (Fig. 4). For the definitive erythrocyte nuclear extracts, complex C3 was the predominating complex, suggesting that most of GATA-1 was associated with other proteins. The primitive erythrocyte nuclear extract formed a higher relative amount of complex C1 than did the definitive erythrocyte nuclear extracts. The abundance of GATA-1 is much greater in primitive than in definitive erythrocytes [5]. Thus, the amount of GATA-1 of primitive erythrocytes may exceed that of the GATA-1 binding proteins. However, we have noted that, despite the inclusion of protease inhibitors in the isolation buffers, the GATA-1 proteins of primitive cell nuclear extracts were more degraded than those from the other nuclear preparations. We cannot exclude the possibility that some of the GATA-1 binding proteins were degraded, resulting in the lowering of the levels of these proteins. Nevertheless, these results show that GATA-1 multiprotein complexes were present in primitive erythrocytes, which express embryonic ρ and ε β-globin genes, and in definitive red blood cells which express the adult β-globin genes.

4. Discussion

The results of the UV-crosslinking experiment provide further evidence that GATA-1 is the only DNA-binding protein of the complexes which are formed with adult chicken immature erythrocyte nuclear extracts and the GATA sequence of the histone H5 enhancer. Complex C1 is formed by the interaction of GATA-1 with its target DNA sequence, while complexes C2–C5 are generated by several non-DNA-binding proteins associating with GATA-1.

GATA-1 multiprotein complexes bound to every variant GATA sequence we have tested. However, the nucleotides flanking the GATAA sequence influenced the binding affinities of GATA-1 and GATA-1 multiprotein complexes. The GATA sequences of the oligonucleotides

Table 1
GATA sequences in regulatory elements of erythroid specific genes

Source	Abbreviation	Sequence	Ref.
Chicken histone H5 enhancer	H5 enh	GAGGCTGGAGATAACAGTGCGG	[9]
Chicken β^A-globin enhancer (+ 1,888 to + 1,910)	βG enh	CAGGTTGCAGATAAACATTTTG	[10]
Chicken β^A-globin promoter	TATA/GATA	GGCGGAGGCGATAAAAGTGGGG	[11]
Chicken ρ-globin promoter (−200 to −206)	ρ-promoter	GGACAGCAAGATAAGGGCTGCT	[5]
Mouse α1-globin promoter (−189 to −174)	αG2 promoter	GGGGCAACTGATAAGGATTCCC	[12]

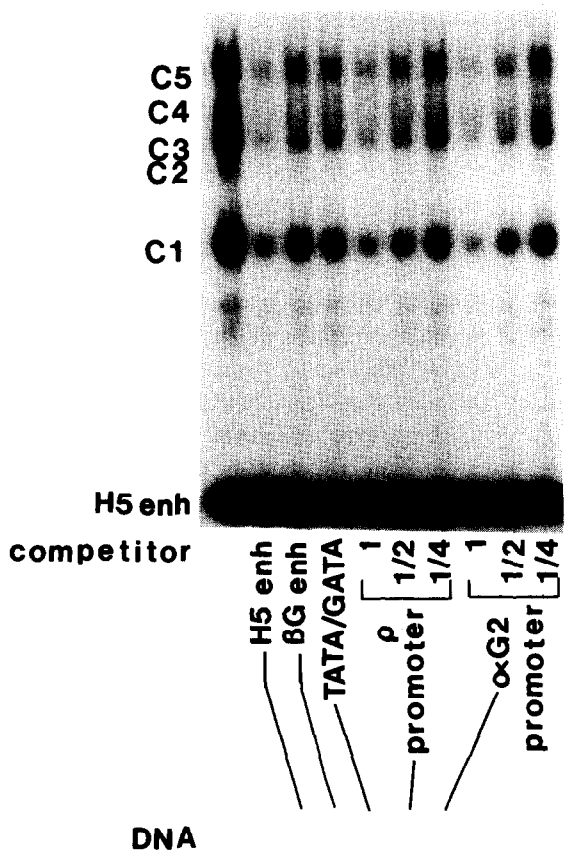

Fig. 3. Relative affinities of GATA-1 and GATA-1 multiprotein complexes for GATA-binding sequences found in regulatory elements of erythroid-specific genes. H5 enh oligonucleotide was end-labelled (0.025 ng) and allowed to bind to adult chicken immature erythrocyte nuclear extracted proteins (2.5 μg) in the absence and presence of competitor DNA. The left lane does not have competitor DNA added and is a control reference lane. The oligonucleotides, which are described in Table 1 and used for competitions, are shown at the lower edge of each lane. Each competitor was used at 2.5 molar excess over the labelled H5 enh oligonucleotide (designated 1 for ρ promoter and αG2 promoter). The ρ promoter and αG2 promoter oligonucleotides were also used at 1.25 (designated 1/2) and 0.625 (designated 1/4) molar excess of labelled H5 enh oligonucleotide. 50 ng of each competitor oligonucleotide was also electrophoresed in a 12% polyacrylamide gel (1 × TBE buffer), and the gel was stained with ethidium bromide as shown in the lower portion of the figure. The oligonucleotides are as indicated.

αG2 promoter, ρ promoter and H5 enh bound both GATA-1 and the GATA-1 multiprotein complexes with high affinity, while the GATA motif of oligonucleotides TATA/GATA and βG enh were of low affinity. Further, our results suggested that the proteins bound to GATA-1 did not alter GATA-1's relative affinity for any variant GATA sequence. Plumb et al. [12] also observed that the βG enh oligonucleotide was less efficient than the αG2 promoter sequence at binding GATA-1.

Recently, Whyatt et al. [14] employed a random oligonucleotide procedure to determine the binding specificity of GATA-1. The preferred GATA motif was AGATAGGGG. Taking G of GATA as position 0, there was a selection against G at position −1, T at position + 4, and C/A at position + 5. Similarly, an analysis of GATA consensus sites found in vivo suggests that the preferred binding site for GATA-1 is A/T GATAAGG, with a bias against T at position −2, G/C at position −1, T/C at position + 4, T at position + 5, and T at position + 7 [14]. Based on the above observations and considering the sequence of the variant GATA oligonucleotides from positions −1 to + 7, the predicted order of binding strengths for GATA-1 would be αG2 promoter, ρ promoter > H5 enh > TATA/GATA > βG enh. This predicted order or relative binding affinities agrees well with our experimental observations.

The specialized β-globin promoter TATA sequence is also present at position −30 in the ρ- and ε-globin gene promoters. Emerson and colleagues have proposed that the binding of GATA-1 to the −30 GATA/TATA establishes a protein–protein linkage between the promoter and enhancer [11]. Once this communication between these two elements forms, GATA-1 is displaced by a TFIID/adaptor complex. Our results suggest that in both primitive and definitive erythroid cells the GATA-1 mul-

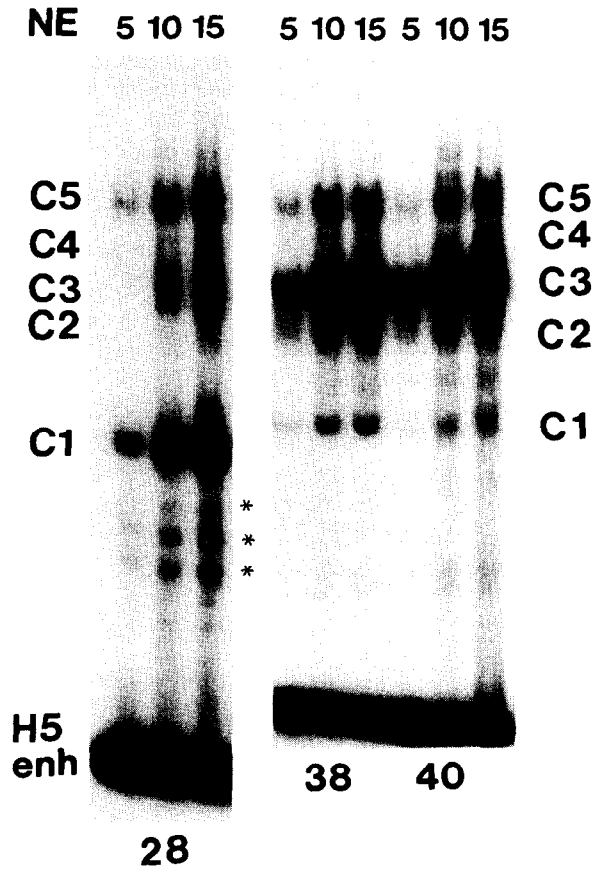

Fig. 4. GATA-1 multiprotein complexes are present in nuclear extracts of chicken primitive and definitive erythrocytes. Protein (NE; 5, 10 or 15 μg) extracted from the erythrocyte nuclei of each embryo stage (stages 28, 38 and 40) was incubated with 1 ng of labelled H5 enh oligonucleotide. The asterisks mark complexes that were presumably generated by degraded forms of GATA-1. Protein–DNA complexes are indicated.

tiprotein complex and not GATA-1 alone would bind to the specialized TATA sequence of the ρ-, ε- and β-globin gene promoters. The low affinity of this sequence for the GATA-1 multiprotein complex would allow its displacement by basal transcription factors.

Erythroid lineage-specific differences in the levels of the transcription factors GATA-1 and Sp1 are thought to play a role in the developmental switch in ρ-globin gene expression [5]. Two elements in the ρ-globin gene promoter seem important in the activation of transcription of this gene, a GATA site and a low-affinity Sp1-binding site (GGGGTGGGG). Similar to the ρ-globin gene promoter, the histone H5 enhancer has one high-affinity GATA-1-binding site. But unlike the ρ-globin gene, the histone H5 gene is expressed in both primitive and definitive erythrocytes. Besides the GATA site, the histone H5 enhancer has four Sp1-binding sites, one of medium affinity and three of low affinity [8]. We propose that the reduced levels of Sp1 and not GATA-1 in definitive erythrocytes are responsible for the inactivation of the ρ-globin gene, and the multiple Sp1 sites in the histone H5 enhancer ensure that Sp1 is recruited to one or more of the Sp1-binding sites.

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