The erythroid-specific protein cGATA-1 mediates distal enhancer activity through a specialized β-globin TATA box

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The erythroid-specific protein cGATA-1 regulates the chick β-globin gene through GATA sequences present at the canonical TATA location in the promoter as well as in the distal 3' enhancer. We have analyzed β-globin transcription in transfected erythroid cells and in erythroid extracts to determine whether cGATA-1 binding at -30 regulates promoter or enhancer activity. The interaction of both cGATA-1 and TFIID at different times with the -30 GATA site is required for efficient β-globin expression in vivo, and the GATA enhancer site can functionally replace the TATA element in the β-globin promoter. TFIID initiates transcription in vitro by complexing with adaptor proteins and displacing cGATA-1 from the -30 GATA site. Mutations that abolish TFIID binding to the -30 GATA box inactivate the promoter, whereas elimination of cGATA-1 binding to this site selectively diminishes enhancer-dependent transcription. We propose that interaction of CGATA-1 with the distal 3' enhancer and the specialized TATA box confers erythroid specificity to the initiation complex by mediating promoter-enhancer communication. Thus, one mechanism of action for tissue-specific proteins that recognizes noncanonical TATA motifs is to enable TFIID to be regulated by distal control elements. In this way, the initiation complex can be responsive to specific regulators that may not recognize a canonical TFIID-TATA structure.

Key Words: Promoter–enhancer interaction; TATA box/TFIID; erythroid transcription factor, GATA-1; β-globin gene transcription

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The chick β-globin gene is part of a multigene family whose individual members are expressed at different stages of erythroid development. Transcription of the embryonic globin genes occurs only in primitive red blood cells (RBCs), whereas expression of the adult globin genes is restricted to definitive erythrocytes. This switch in red cell lineages occurs by about day 5 of chick embryogenesis [Bruns and Ingram 1973; Brown and Ingram 1974; Chapman and Tobin 1979]. The regulation of β-globin gene activity is achieved primarily through changes in chromatin structure [Stalder et al. 1980; McGhee et al. 1981] and the interaction of specific DNA-binding proteins with the 5' promoter and 3' enhancer [Emerson et al. 1985, 1987, 1989; Plumb et al. 1986; Evans et al. 1988; Lewis et al. 1988). These two control regions bind multiple proteins, consisting of tissue-specific and general factors, whose composition changes during erythroid maturation. Recently, an in vitro transcription system was devised using extracts from primary RBCs isolated at different stages of embryonic development. In this soluble system, the β-globin gene is expressed in a manner similar to that observed with the endogenous gene in red cell nuclei. These experiments demonstrated that transcriptional regulation is achieved by a combination of activator proteins whose concentration is highest in expressing RBCs of the appropriate developmental stage. As erythroid maturation proceeds, the activity of a potent repressor, PAL, increases severalfold and shuts off β-globin expression by binding the promoter and counteracting the effects of the activator proteins [Emerson et al. 1989]. Chromatin reconstitution experiments have shown that the interaction of these multiple transcription factors with the β-globin promoter induces a nucleosome hypersensitive site similar to the developmentally regulated structure observed in definitive RBCs [Emerson and Felsenfeld 1984]. Thus, several important aspects of β-globin gene regulation can be reproduced by using in vitro systems.

One critical control region that has not been analyzed in vitro is the 3' enhancer. This region is required for β-globin expression in transfected RBCs [Hesse et al. 1986] yet is not functional in the soluble transcription system [B.M. Emerson, unpubl.]. Enhancer activity in vivo is dependent on DNA sequences that interact with several proteins, including the erythroid-specific enhancer-binding factor cGATA-1 [Emerson et al. 1987; Evans et al. 1988; Reitman and Felsenfeld 1988]. This
protein has been purified (37 kD) and recently cloned from murine [mGATA-1], chick [cGATA-1], and human [hGATA-1] erythroid cells [Evans and Felsenfeld 1989; Tsai et al. 1989; Trainor et al. 1990]. GATA-1 is highly tissue specific, and its binding site is found in the control regions of many erythroid genes [Evans et al. 1988; Plumb et al. 1989]. Experiments with chimeric mice have shown that mutations in the GATA-1 gene result in a block in erythroid maturation. GATA-1, therefore, may represent a "switch" protein involved in the differentiation of the erythroid lineage [Pevny et al. 1991].

Recent studies indicate that GATA-1 is part of a multigene family [Wilson et al. 1990; Yamamoto et al. 1990]. One member of this family, GATA-3, is highly expressed in T lymphocytes and brain. This protein was first implicated in the regulation of T-cell-specific genes because the GATA consensus-binding site is located in the enhancer regions of the α and δ chains of the T-cell receptor [TCR [Ho et al. 1989, 1991; Winoto and Baltimore 1989, Redondo et al. 1990]]. More recent functional experiments demonstrate that both mouse and human GATA-3 can trans-activate expression of the human TCR β gene through the GATA sequence in the enhancer [Ko et al. 1991]. Thus, the GATA family of proteins plays a critical role in regulating tissue-specific enhancer activity in several different cell types. As a first step in understanding the mechanism of action of these important regulatory proteins, we have analyzed the function of the erythroid-specific cGATA-1 in β-globin gene expression.

Here, we show that purified cGATA-1 binds specifically to both the 3′ enhancer and to the specialized TATA sequence [GATA at −30] in the β-globin promoter, suggesting that promoter–enhancer communication may be mediated through this element. Mutations in the −30 GATA box that differentially abolish the binding of either cGATA-1 or TFIID have been analyzed in vivo and in vitro. These results indicate that TFIID is necessary for transcriptional initiation, and cGATA-1 regulates the ability of the distal enhancer to activate the promoter. Both proteins function separately through the same DNA-binding site and can displace each other, depending on their relative concentrations. Moreover, we find that non-DNA-binding proteins, or adaptors, are required to mediate this effect. Thus, a critical step in the tissue-specific regulation of the β-globin gene is the establishment of enhancer–promoter interaction mediated, in part, by cGATA-1 bound to −30. Once this interaction is stable, TFIID in combination with adaptor proteins can displace cGATA-1 from the −30 GATA site to form an active initiation complex.

Results

cGATA-1 interacts with both the promoter and enhancer regions of the chick β-globin gene

To analyze how the distal β-globin enhancer may affect promoter function, we initially performed DNase protection experiments on the promoter region using purified cGATA-1. Previous studies have shown that at least eight proteins interact with this control region [Lewis et al. 1988; Emerson et al. 1989], including the TATA box. When purified cGATA-1 was analyzed on the β-globin promoter in DNase protection experiments, only the GATA sequence at −30 was bound by protein [Fig. 1, right]. The protected region, CCGAGGGCATAAA, contains the core sequence GATAAA, which is also present in the GATA-1-binding site within the β-globin enhancer between +1891 and +1906 [Fig. 1, left]. Thus, cGATA-1 interacts with a GATA element in both the enhancer and promoter at the canonical TATA box position. To confirm this observation, we found that cGATA-1 binding to its 5′ and 3′ sites was specifically abolished in the presence of competing oligonucleotides to either the promoter or enhancer GATA sequences (data not shown). This supports the idea that these two sequences are recognized by the same DNA-binding domain within the cGATA-1 protein.

Conversion of the −30 GATA sequence to a canonical TATA box abolishes cGATA-1 binding

We examined the binding ability of cGATA-1 on β-globin promoters containing mutated sequences in the −30 GATA box by a gel mobility-shift analysis. Interaction with the normal sequence at −30 (GAGG-GATAAAAGT) was compared with that of a GG mutated sequence (GAGGCCAGGAAGT) and a canonical TATA box (GAGGTATATAAGT). As shown in Figure 2A, the cGATA-1 protein binds specifically to the GATA sequence (lanes 2,3) whereas no interaction is observed with either the β-globin TATA (lane 8), adenovirus TATA (Adeno TATA; lane 10), or GG (lane 12) oligonucleotides. Moreover, in oligonucleotide competition experiments the cGATA-1/GATA DNA complex is inhibited by GATA (lane 4) but not by TATA (lane 5) or GG (lane 6) oligonucleotides. Thus, a mutation of the GATA sequence to a canonical TFIID recognition site completely abolishes the binding of cGATA-1. This analysis was also used to examine the interaction of TFIID with these oligonucleotides, as shown in Figure 2B. Through the use of bacterially expressed yeast TFIID, binding is observed to both the adenovirus major late promoter (AdMLP) [lanes 2,3] and mutated β-globin TATA (lane 8) sequences. In contrast to cGATA-1, however, cloned TFIID binds only weakly to the β-globin GATA oligonucleotide (lane 10) and is completely abolished on the oligonucleotide containing the GG mutation [lane 12]. TFIID interaction with the β-globin TATA sequence is specifically inhibited with competitor oligonucleotides to the TATA site [lane 4], whereas competition with either GATA [lane 5] or GG [lane 6] oligonucleotides has no effect on TFIID binding. Taken together, these experiments indicate that conversion of the GATA site to a canonical TATA box specifically inhibits the interaction of cGATA-1 and that mutation of TATA to GATA severely weakens the binding of cloned TFIID. The interaction of both cGATA-1 and TFIID is eliminated by mutation of GATA to GAGG.
cGATA-1 binding to the −30 GATA box is required for efficient enhancer-dependent transcription

To examine the functional role of cGATA-1 interaction with the promoter, we compared the activity of the normal promoter sequence at −30 with that of the GG and TATA mutations, which no longer bind cGATA-1 by transfection into primary cultures of chick embryonic RBCs. In this analysis the modified and unmodified β-globin promoters were linked to the chloramphenicol acetyltransferase (CAT) gene and the 3′ β-globin enhancer. Conditions for transfection were according to the procedure of Jackson et al. (1989). As shown in Figure 3, β-globin–CAT constructs containing the unmodified promoter and 3′ enhancer (AcateE30) are efficiently expressed in 11-day chick erythrocytes relative to a Rous sarcoma virus promoter–CAT gene (RSV–CAT) control. If the 3′ enhancer is deleted from these β-globin–CAT constructs, leaving only a functional promoter (Acate), transcription is almost completely abolished (6.1%). CAT constructs having a functional enhancer but a promoter carrying the GG mutation (AcateE/GG) show severely reduced transcriptional efficiency (16.8%), presumably because neither cGATA-1 nor TFIID can bind to this sequence (Fig. 2). Interestingly, when the β-globin −30 GATA sequence is mutated to a canonical TATA box (Acate/TATA), expression levels of β-globin–CAT constructs are decreased by 70% relative to the wild-type promoter (AcateE30) even though TFIID binds strongly to this site (Fig. 2B). This result indicates that TFIID binding to a TATA element is not sufficient for high levels of enhancer-dependent β-globin expression and that cGATA-1 binding at −30 is required to mediate this effect.

The cGATA-1 enhancer-binding site can function as a TATA element when placed in the promoter

Because cGATA-1 can interact at sites in both the promoter and enhancer, we constructed β-globin templates containing the enhancer GATA-1-binding site (TTGCA-GATAAACAT) in place of the GATA sequence in the promoter. p-Globin genes containing this substitution were examined by DNase protection and by transfection into primary erythroid cells. The protein interaction pattern obtained by DNase protection indicates that cGATA-1 can efficiently bind to the GATA enhancer sequence when placed at −30 and that this complex does not alter the binding of any other promoter factor [data not shown]. To assess whether the site through which cGATA-1 regulates the enhancer can also direct transcription, β-globin–CAT constructs containing the GATA(Enh)-substituted promoter and the 3′ enhancer were analyzed for expression in 11-day chick erythrocytes. As shown in Figure 3, these constructs (AcateE/RIV) are expressed with 63% the efficiency of those containing the unmodified GATA box (AcateE30). These results demonstrate that a tissue-specific enhancer element can functionally
Figure 2. Gel mobility-shift analysis of cGATA-1 and TFIID interaction with GATA and TATA sequences. Affinity-purified cGATA-1 and bacterially expressed yeast TFIID were incubated with 32P-end-labeled oligonucleotides containing modifications of the -30 GATA sequence: TATA (TATAAA) and GG (GAGGAAA). Oligonucleotides containing the canonical TATA sequence from the AdMLP were also used. (A) cGATA-1 protein binds specifically to GATA sequences. Two nanograms of cGATA-1 protein was incubated with the following 5' 32P-labeled oligonucleotides (0.025-0.05 ng): GATA (lanes 3-6; lane 2 contained 1 ng of cGATA-1); TATA [lane 8]; Ad TATA [lane 10]; and GG [lane 12]. (Lanes 1, 7, 9, 11) Reactions with no protein. Oligonucleotide competition experiments [lanes 4-6] were performed by preincubating cGATA-1 protein with 7.5 ng of unlabeled oligonucleotide to GATA (lane 4), TATA (lane 5), or GG (lane 6) sequences. (B) TFIID binds specifically to TATA sequences. Five nanograms of yeast TFIID was incubated with the following 5' 32P-labeled oligonucleotides (0.025-0.05 ng): Ad TATA (lanes 3-6; lane 2 contained 2.5 of ng TFIID), TATA [lane 8], GATA [lane 10], and GG [lane 12]. (Lanes 1, 7, 9, 11) Reactions with no protein. Oligonucleotide competition experiments [lanes 4-6] were performed by preincubating TFIID with 7.5 ng of unlabeled oligonucleotide to TATA (lane 4), GATA (lane 5), or GG (lane 6) sequences.

replace the β-globin TATA box and mediate the activity of erythroid TFIID.

The lower transcriptional activity of AcatE/RIV, relative to AcatE30, suggests that although cGATA-1-binding sites in both the promoter and enhancer can function as TATA elements, they are not absolutely equivalent. For example, TFIID may bind more tightly to the wild-type GATA environment at -30. Mutating this site to the GATA enhancer sequence may diminish this interaction and result in a slightly lower level of expression.

Figure 3. In vivo activity of wild-type and modified β-globin promoters direct the expression of bacterial CAT genes in transfected primary 11-day RBCs. Primary RBCs (3 x 107 to 5 x 107) were transfected with 3 μg/ml of DNA as described by Jackson et al. (1989). Following a 48-hr incubation at 37°C, the cells were processed and assayed for CAT activity. [RVS-CAT] Plasmid containing the RSV LTR directing CAT expression. [Ac] Wild-type β-globin promoter (-1000 to +43) linked to the CAT gene with a deleted 3' β-globin enhancer. [AcE30] Wild-type β-globin promoter linked to the CAT gene with the 3' β-globin enhancer (+1814 to +2231]. [AcE/GG] AcE30 with GG mutation at the -30 GATA box. [AcE/RIV] AcE30 with cGATA-1 enhancer sequence (region IV) substitution at the -30 GATA box. [AcE/TATA] AcE30 with TATA mutation in the -30 GATA box. The relative activity for each construct was determined by dividing the percent [14C]chloramphenicol conversion for each mutant by the percent conversion for the wild-type promoter with enhancer (pAcE30). The average relative activities were calculated from four experiments containing three to four replicates of each construct.
However, the observation that the GATA(Enh)-substituted construct is more active than one containing a normal TATA box (AcATETATA) further supports the notion that cGATA-1 must interact with both the enhancer and the -30 GATA site for efficient expression to occur.

cGATA-1 binding at -30 is not required for β-globin promoter activity in vitro

β-Globin promoter mutations that decrease CAT expression in transfected erythroid cells (Fig. 3) may do so by either lowering promoter activity itself or by affecting the ability of this element to respond to the distal enhancer. To discriminate between these two possibilities, we examined β-globin templates containing mutations in the -30 GATA box for transcriptional activity in vitro. In most in vitro systems, gene expression levels are determined solely by promoter strength because distal enhancer elements are not functional. In our case, the chick β-globin gene is transcribed in erythroid extracts with equal efficiency in the presence or absence of its 3′ enhancer. Therefore, the in vitro transcription levels obtained from β-globin genes mutated at the -30 GATA box are a measure of promoter activity rather than promoter–enhancer interactions. In contrast, expression levels obtained from genes transfected into erythroid cells are dependent upon both promoter strength and enhancer activity.

In vitro transcription experiments were carried out using erythroid extracts obtained from 11-day chick embryonic RBCs as described previously [Emerson et al. 1989]. The parental template used in these studies contains the entire 1.5-kb chick β-globin gene with 0.4 and 1.5 kb of 5′- and 3′-flanking sequences, respectively. These sequences comprise the 5′- and 3′-nuclease hypersensitive regions as well as the 3′ enhancer. A 17-bp BamHI–XmaI linker was inserted at +46 in the 5′-untranslated leader to distinguish in vitro-synthesized globin RNA from the endogenous globin message present in the RBC transcription extracts. All reactions were analyzed for β-globin synthesis by the S1 nuclease technique, using a probe that detects both endogenous and newly generated transcripts.

As shown in Figure 4, β-globin genes containing the GG mutation, which abolishes both cGATA-1 and TFIIID binding to the -30 GATA box [Fig. 2], are transcriptionally inactive in 11-day RBC extracts (lane 2), whereas high levels of RNA synthesis are obtained with the unmodified template (lane 1). In contrast, when the -30 GATA box is mutated to a normal TATA sequence, which no longer interacts with cGATA-1 but binds TFIIID with much higher affinity [Fig. 2], transcription levels remain the same (lane 4). This suggests that when TFIIID can interact efficiently at -30 through a TATA sequence, cGATA-1 is not required for promoter activity. This presumably reflects the greater affinity of TFIIID for TATA than for GATA sequences [Fig. 2]. β-Globin genes containing the cGATA-1 enhancer-binding site (region IV) in place of the -30 GATA sequence can direct correctly initiated transcription in erythroid extracts (lane 3), although to a lower extent than that observed in transfected erythroid cells (Fig. 3). In all β-globin constructs the 3′-enhancer region can be deleted with no effect on expression levels in erythroid transcription extracts (data not shown). This is consistent with the observation that distal enhancer regions are not normally required in in vitro transcription systems using naked DNA templates.

A comparison of transcriptional levels obtained in vivo (Fig. 3) and in vitro (Fig. 4) indicates that the inactivity of β-globin genes containing the -30 GG mutation is most likely because TFIIID is unable to bind the promoter and there is no initiation. Unlike the situation in vivo, where low-level transcription is observed with the GG mutation (Fig. 3), there is no activity in vitro, presumably because the enhancer does not function under these conditions and expression is entirely promoter dependent. Thus, the GG mutational effects can be attributed to an inactive promoter. In contrast, β-globin genes containing the TATA mutation are much more active in vitro than in vivo, relative to the normal GATA template. Because this sequence change does not lower the activity of the promoter, the decreased expression in vivo must be the result of a disruption in promoter–enhancer interaction, which requires the binding of cGATA-1 at -30.

TFIIID regulates β-globin promoter activity through the -30 GATA box in vitro

Because cGATA-1 and TFIIID must interact through the
same initiation element at −30, we asked what relation these proteins have to one another and whether cGATA-1 itself can function as TFIID or act cooperatively with it. To address these questions we used an in vitro system that relies on the addition of TFIID to complement transcription in heat-inactivated HeLa extracts (Nakajima et al. 1988). For these studies, rabbit reticulocyte-translated human TFIID (hTFIID) and cGATA-1 were used as protein sources. As shown in Figure 5, heat-inactivated HeLa extracts (ΔT HeLa) by themselves [lane 2] or in combination with reticulocyte control lysate [lane 3] fail to complement transcription on β-globin genes containing the −30 GATA box. However, when these extracts are programmed with in vitro-translated hTFIID, β-globin genes are expressed efficiently [lanes 4,5]. In contrast, transcription reactions complemented with in vitro-translated cGATA-1 fail to express β-globin genes [lanes 6,7] or stimulate TFIID activity [lane 8] even though this protein binds the −30 GATA sequence with high affinity [Fig. 2A]. As a control, in vitro-translated Jun protein was also tested and found to have no TFIID stimulatory [lane 9] or complementing [lane 10] activity on β-globin gene templates. These results indicate that cGATA-1, by itself, does not function as an erythroid-specific TFIID factor when bound to the β-globin initiation element.

Because HeLa extracts that are heat inactivated can be fully reconstituted for transcriptional activity by the addition of a single protein, cloned TFIID, this demonstrates that TFIID has been selectively inactivated and is the only protein required for complementation of β-globin transcription in this assay. This indicates that the β-globin promoter is TFIID dependent even though it contains a noncanonical TATA box that interacts with an erythroid-specific protein, cGATA-1. The observation that cGATA-1 cannot restore transcription in an assay that is specific for TFIID activity suggests that it serves a different function at the initiation site. Because the GATA protein is part of a multigene family, the members of which are presumably present in most cell types, it is formally possible that they bind to the β-globin promoter and activate transcription in HeLa extracts. However, these nonerythroid GATA proteins do not obviate the requirement for TFIID because they are not sufficient to restore transcription if TFIID has been inactivated.

Taken together, these results suggest that TFIID is a necessary component of β-globin promoter activation in vitro and that the major role of cGATA-1 is not to function as a direct transcriptional activator when bound at −30 but, rather, to mediate enhancer activity, potentially by establishing an interaction between the distal enhancer and promoter control elements.

TFIID requires adaptor proteins to interact with the −30 GATA box and to displace cGATA-1 from this site

In erythroid cells TFIID must apparently recognize a cGATA-1/DNA complex at −30. To determine whether these two proteins can form a stable association with each other on GATA-binding sites we conducted gel mobility-shift experiments using affinity-purified cGATA-1 and bacterially expressed hTFIID. As shown in Figure 6A, hTFIID binds very weakly to 32P-labeled GATA oligonucleotides [lane 3] in contrast to cGATA-1 [lane 2]. When both proteins are combined, no detectable mobility shift of the cGATA-1/GATA complex is observed [lanes 4,5], indicating that cloned hTFIID does not form a stable complex with cGATA-1. However, as shown in Figure 6B, when hTFIID is added to a non-DNA-binding protein fraction from erythroid cells that is likely to contain factors that mediate protein–protein interactions, a stable hTFIID complex is formed on GATA oligonucleotides [lanes 4–6]. This complex is higher in mobility than hTFIID alone [lane 2], indicating that at least one protein in this “adaptor” fraction is associated with hTFIID and greatly increases its affinity for GATA sites. The adaptor fraction displays minimal DNA-binding activity itself and contains little, if any, cGATA-1 protein [lane 3] even over a broad concentration range [data not shown]. This demonstrates that cGATA-1 is not required for hTFIID to interact efficiently with GATA sites. Interestingly, when the adaptor fraction is combined with cGATA-1, no change in mobility of the cGATA-1/GATA complex is observed but there is a significant decrease in cGATA-1 binding [lanes 7–9]. Moreover, the addition of cGATA-1 to the hTFIID–adaptor
Figure 6. Gel mobility-shift analysis of cGATA-1 and TFIID displacement. Affinity-purified cGATA-1 and bacterially expressed hTFIID were incubated with 32P-end-labeled oligonucleotides containing the −30 GATA sequence, unless otherwise indicated, using the conditions described in Fig. 2. (A) cGATA-1 and TFIID do not form a complex together on GATA DNA sequences. The following proteins were incubated with 32P-labeled GATA oligonucleotides: no protein (lane 1); 2 ng of cGATA-1 (lane 2); 5 ng of hTFIID (lane 3); 5 ng of hTFIID plus 2 ng of cGATA-1 (lane 4); 5 ng of cGATA-1 (lane 5). (B) TFIID binds efficiently to GATA DNA in the presence of adaptor proteins. 32P-labeled GATA oligonucleotides were incubated with the following proteins: 2 ng of cGATA-1 (lane 1); 5 ng of hTFIID (lane 2); 20 ng of DNA-cellulose/25 mM (NH4)2SO4 fraction (DC/25 mM) (lane 3); 5 ng of hTFIID plus 10, 20, and 40 ng of DC/25 mM (lanes 4–6); 2 ng of cGATA-1 plus 10, 20, and 40 ng of DC/25 mM (lanes 7–9); 5 ng of hTFIID and 2 ng of cGATA-1 plus 20 ng of DC/25 mM after incubation for 1 hr at 0°C with antisera to hTFIID (lane 13) or antisera to an unrelated transcription factor, HIP (lane 14). (C) TFIID and adaptor proteins displace cGATA-1 from −30 GATA DNA. The following proteins were incubated with 32P-labeled GATA oligonucleotides: 2 ng of cGATA-1 (lane 1); 20 ng of DC/25 mM plus 5 ng of hTFIID, 5 ng of Sp1 or 6 ng of Jun (lanes 2–4); 20 ng of DC/25 mM and 5 ng of hTFIID plus 2, 5, and 7 ng of cGATA-1 (lanes 5–7). The following proteins were incubated with 32P-labeled TATA oligonucleotides: 5 ng of hTFIID (lane 1); 2 ng of cGATA-1 (lane 2); 20 ng of DC/25 mM (lane 10); 20 ng of DC/25 mM plus 5 ng of hTFIID (lane 11); 20 ng of DC/25 mM and 5 ng of hTFIID plus 2 ng of cGATA-1 (lane 12). The following proteins were incubated with 32P-labeled GG oligonucleotides (lane 13) or SSE oligonucleotides (lane 14): 20 ng of DC/25 mM and 5 ng of hTFIID plus 2 ng of cGATA-1.

Because the decrease of cGATA-1 binding in the presence of the hTFIID-adaptor complex was so striking, we varied the concentration of cGATA-1 in these reactions to examine the dynamics of interaction between these two complexes. At low cGATA-1 concentrations, the hTFIID-adaptor complex is present in high abundance while cGATA-1 binding is effectively eliminated [Fig. 6C; cf. lanes 1 and 5]. As the concentration of cGATA-1
increases, a change in the relative abundance of the hTFIID–adaptor complex and the cGATA-1/GATA complex is observed [lane 6], such that at the highest cGATA-1 levels the hTFIID–adaptor complex is negligible while the cGATA-1/GATA complex is efficiently formed [lane 7]. These results suggest that there is an active displacement of cGATA-1 or TFIID binding and that the protein occupancy of GATA sites is determined by the relative concentrations of cGATA-1, TFIID, and adaptor proteins. This displacement is more likely the result of a competition for adaptor proteins between cGATA-1 and TFIID rather than a competition for GATA-binding sites, as the reactions are performed in DNA excess.

We then compared the binding behavior of these proteins on canonical TATA sites. As shown in Figure 6C, neither cGATA-1 [lane 9] nor the adaptor fraction [lane 10] binds to TATA oligonucleotides, and cloned hTFIID binds weakly [lane 8]. However, when hTFIID and the adaptor proteins are combined, a high-molecular-weight complex is formed [lane 11] that comigrates with the hTFIID–adaptor complex formed on GATA oligonucleotides [lane 2]. The addition of cGATA-1 has no effect on the hTFIID–adaptor complex [lane 12], and the complex does not form on nonspecific DNA sites such as GG [lane 13] or SSE [lane 14]. The hTFIID and adaptor proteins bind much more efficiently on TATA than on GATA sites, and the observation that the two complexes comigrate supports, although does not conclusively prove, the idea that the protein composition on the two DNA sites is the same. Thus, at this time, there is no evidence to invoke the existence of different adaptors associating with TFIID on GATA and TATA boxes, although this remains a possibility. The further characterization of the adaptor proteins is currently in progress.

cGATA-1 mediates β-globin promoter–enhancer interaction

A summary of the binding data presented in the preceding section is shown in Figure 7. The erythroid-specific protein cGATA-1 regulates β-globin gene expression by binding to a distal, 3′ enhancer and to a specialized TATA box in the promoter. Although the exact mechanism is unknown, we speculate that cGATA-1 at the −30 GATA site forms a complex with itself or one of several other proteins bound to the enhancer through DNA loop formation. A model similar to this was originally proposed by Gallarda et al. [1989]. This structure is recognized by TFIID, in combination with adaptor proteins, and cGATA-1 is displaced from the −30 GATA box to allow transcriptional initiation. The role of cGATA-1 interaction with a site that mediates TFIID function may be to enable the distal enhancer to regulate the transcriptional initiation complex. Because cGATA-1 is a tissue-specific protein, this requirement imposes an erythroid-specific level of control on enhancer-dependent β-globin gene expression. The proposed structure between the promoter and enhancer at the initiation site results in a highly active promoter and an efficient mechanism by which the enhancer can activate expression. If the −30 GATA box is modified to a canonical TATA sequence, the binding affinity of the TFIID–adaptor complex increases but cGATA-1 cannot interact with this site. This does not alter the efficiency of the promoter, but enhancer-dependent expression is markedly decreased. This suggests that the high-affinity interaction of cGATA-1 at −30 is critical for establishing promoter–enhancer interaction prior to the formation of an active initiation complex.

Discussion

The tissue-specific DNA-binding protein cGATA-1 interacts with the control regions of many erythroid-specific genes [Plumb et al. 1989], suggesting that it has a critical role in their regulation. This protein has been characterized in RBCs from several different species [Evans and Felsenfeld 1989; Tsai et al. 1989] and could represent a switch factor that is involved in the determination or maintenance of the erythroid phenotype. In these studies we examined the mechanism of action of cGATA-1 on the transcriptional regulation of the chick β-globin gene. We present evidence that cGATA-1 regulates both the promoter and the distal, 3′ enhancer through interaction with the TATA box. The β-globin gene family contains a specialized TATA sequence at −30 (GATAAA in chick and CATAAA in human). cGATA-1, purified from chick erythrocytes, interacts
with the GATA sequence in the chick β-globin enhancer as well as in the promoter. Modifications of the −30 GATA sequence that eliminate cGATA-1 but not TFIID binding have no effect on promoter activity, as measured by in vitro transcription using chick erythroid extracts, but significantly decrease enhancer-dependent transcription in transfected chick embryonic RBCs. This indicates that cGATA-1 does not function as a transcriptional activator when bound to the −30 GATA box but is instead required to mediate distal enhancer activation of the promoter. This presumably occurs through the formation of a DNA loop between the two control regions. Interestingly, the cGATA-1-binding site within the enhancer functions as a TATA box when placed at the canonical location in the β-globin promoter. Thus, the protein–DNA complex that regulates a tissue-specific enhancer can also function through a specialized TATA element. Moreover, cGATA-1-binding sites in each control region must be functional for the β-globin gene to be expressed. Mutations in the cGATA-1 site of the enhancer have been shown to severely reduce the expression of β-globin CAT constructs (Reitman and Felsenfeld 1988).

Both TFIID and cGATA-1 function through the −30 GATA box and are necessary components of enhancer-dependent β-globin transcription in erythroid cells. Our data demonstrate that these two proteins do not form a complex together but occupy GATA sites at different times. Thus, cGATA-1 does not appear to function in a manner similar to the adenovirus protein E1A, which can directly interact with TFIID and mediate activation by other transcription factors (Horikoshi et al. 1991; Lee et al. 1991). The binding of TFIID to the −30 GATA site requires the addition of non-DNA-binding adaptor proteins because cloned hTFIID has very low affinity for GATA sequences. The TFIID–adapter complex then interacts with cGATA-1 and displaces it from GATA-binding sites. At higher protein levels, cGATA-1 can similarly displace the TFIID–adapter complex. This indicates that the occupancy of the −30 GATA box is carefully regulated by the relative concentrations of cGATA-1, TFIID, and adaptor proteins. Whether the adaptors that we describe are similar to the coactivator or adaptor proteins that mediate activation of the basal transcriptional machinery by other regulatory proteins (Berger et al. 1990; Kelleher et al. 1990; Pugh and Tjian 1990; Meisterernst et al. 1991) or represent the class of factors known as TAFs (Dynlacht et al. 1991) remains to be determined. We propose that the −30 GATA box is occupied by cGATA-1 and TFIID at different times to first establish an interaction between the promoter and enhancer and then to form an active transcriptional initiation complex that is enhancer dependent. The purpose of incorporating cGATA-1 into the initiation site through the specialized TATA box may be to enable the TFIID complex to be regulated by the distal enhancer. By this mechanism, erythroid-specific and enhancer-dependent control of the initiation complex is imparted through a specialized TATA box. In support of this, a recent study demonstrated that mutation of the −30 GATA site in the rat platelet factor 4 gene to a canonical TATA element decreased tissue-specific expression in megakaryocytes, perhaps by rendering the promoter less responsive to the megakaryocyte-specific enhancer that is associated with this gene (Ravid et al. 1991).

Compelling evidence for promoter–enhancer interactions occurring through the TATA sequence comes from studies on the Drosophila alcohol dehydrogenase (Adh) gene (Corbin and Maniatis 1989). This gene is controlled by two promoters that are differentially activated by two separate enhancers at distinct developmental stages. Regulation is thought to occur through different TATA motifs within each promoter that determine the specificity of interactions with the appropriate enhancer. The investigators propose that either two separate TATA factors bind to each promoter or that a single TATA factor interacts differentially with the two motifs. Recent experiments have shown that the muscle-specific enhancer upstream of the human myoglobin gene is functional only with the TATA sequence 'TATAAAA', present in the myoglobin promoter. When this sequence is changed to that of the SV40 promoter, 'TATTAT', the myoglobin enhancer no longer activates transcription (Wefald et al. 1990). A similar situation may exist in the chick β-globin gene if the cGATA-1/GATA complexes in the promoter and enhancer are required to mediate functional interaction between these two elements. Our data suggest that a sequence change to TATA destroys this interaction.

The functional distinction of multiple classes of TATA elements has been demonstrated in several studies. For example, experiments involving the human Hsp70 gene have shown that a conversion of the normal TATA sequence to that of the SV40 'TATTAT' results in transcription that is no longer inducible by EIA (Simon et al. 1988). In yeast, two distinct TATA boxes have been identified in the his3 promoter, Tr and Tcr, which appear to interact with different proteins (Chen and Struhl 1988). These TATA elements respond differentially to the transcriptional activators GCN4 and GAL4 such that only the Tr TATA sequence can be induced by the upstream activators (Harbury and Struhl 1989). Recently, the pituitary-specific transcription factor GHF-1 has been shown to regulate expression of the growth hormone gene through a 15-bp region that includes the TATA box (McCormick et al. 1991). Taken together, these examples of TATA box heterogeneity indicate that TFIID activity can be modulated by differential interactions with non-TATA factors that bind to adjacent or overlapping DNA sequences. These factors may change the structure of the initiation complex and facilitate the functional interaction with regulatory proteins that control different levels of gene expression.

We propose that an initial step in β-globin gene expression is the interaction of cGATA-1 bound to the −30 GATA box with cGATA-1, or other proteins, bound to the 3′ enhancer. This interaction may have a critical role in allowing the two control regions to communicate with each other, presumably by DNA loop formation. Once the DNA loop is formed, TFIID and adaptor pro-

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peptides displace cGATA-1 and produce an active initiation complex that is under the control of the erythroid-specific enhancer. Because cGATA-1 is present at all stages of erythroid development, this loop may form in primitive RBCs long before the β-globin gene is expressed. Alternatively, in definitive RBCs this connection between the two control regions could be stabilized by the binding of other proteins to the promoter and enhancer, such as the erythroid stage-specific factor NF-E4 (Gallard et al. 1989). Interestingly, the cGATA-1–enhancer complex is situated between the β- and ε-globin genes and controls their expression at different stages of erythroid development (Choi and Engel 1988; Nicol and Felsenfeld 1988). The NF-E4 protein has been implicated in causing the regulated switch in transcription from the ε- to the β-globin gene by altering the interaction of the β-globin enhancer with the promoters for each of these templates (Gallard et al. 1989). This factor has been shown previously to be an activator of β-globin gene transcription in an erythroid cell-free system (Emerson et al. 1989).

A comparison of TATA elements in the β-globin gene family indicates that both the embryonic, ρ, and adult β-globin promoters contain cGATA-1 binding sites at −30 and not elsewhere in the near vicinity. In contrast, the β-γ-hatching and β-ε-globin genes contain a canonical TATA sequence at −30 and have cGATA-I sites within 60 bp upstream (Dodgson et al. 1983). The α-globin genes are regulated much differently than the β-globin family, because they are expressed throughout erythropoiesis rather than at discrete stages. We suggest that cGATA-1 may regulate erythroid genes having either TATA or GATA sequences at −30 by different mechanisms. For example, in the α1-globin gene, the TFIID initiation complex could be under erythroid-specific control if cGATA-1 is required to activate it from nearby sites. In this case, cGATA-1 would function as a direct activator of TFIID. In genes such as β-, ρ-, and ε-globin, cGATA-1 binds to the −30 GATA element and may affect the activity of TFIID indirectly by enabling it to interact with a distal enhancer. Thus, cGATA-1 is a transcription factor that may have different mechanisms of action depending on where it binds and which proteins it interacts with.

The erythroid GATA-1 protein is part of a multigene family that includes members that also regulate other tissue-specific enhancer regions. For example, GATA-3 is abundantly expressed in T lymphocytes and brain cells (Yamamoto et al. 1990; Ho et al. 1991) and is involved in the control of the TCR β-chain enhancer (Ko et al. 1991). It will be interesting to compare the mechanism by which other members of the GATA family regulate promoter–enhancer activity with that of the erythroid-specific cGATA-1 factor.

Materials and methods

Plasmid constructions

The standard chick β-globin gene template (pUC18ABC/Δ1) used in the in vitro transcription experiments contains the upstream chicken β^A-globin promoter (to −407), a 17-bp insert at position +46 to mark the gene, and 1.5 kb of 3′-flanking sequences, which includes the entire β-globin enhancer region (Emerson et al. 1989). β^A-Globin genes containing modified TATA boxes were derived from this parental plasmid (Δ1). The wild-type GATA box sequence at −30 was mutated from GAGGA in Δ1/GG and to TATAA in Δ1/TATA. The plasmid Δ1/RIV contained the GATA [enhancer] region IV site [TTCGAGATAACCAT, +1899 to +1911] in place of the GATA box sequence [GAGGCAGATAAAAG, −35 to −23] in the promoter. These promoter mutations were constructed by synthesizing 34-bp oligonucleotides (−94 to −10) containing the three sequence changes. The oligonucleotides were synthesized to contain overhanging ends for Apal [5′] and Eagl [3′] restriction sites. Each mutated oligonucleotide was then inserted into the Apal [−94] and Eagl [−10] sites of the parental plasmid pUC18ABC/Δ1 for in vitro transcription templates or into a plasmid, p8A, which contains only β-globin promoter sequences [−407 to +196] for DNase I protection studies.

β-Globin templates used to transfected primary chick erythrocytes were derived from Acate30 with the 3′ β-globin enhancer extending from +1814 to +2231 and the β-globin promoter from −1000 to +43 (Hesse et al. 1986). Acate is identical to Acate30 except that the 417-bp 3′ enhancer fragment is deleted. The RSV–CAT control plasmid contains the RSV LTR linked to the bacterial CAT gene. The plasmids Acate/EG, Acate/TATA, and Acate/RIV contain modified GATA boxes as described for Δ1/GG, Δ1/TATA, and Δ1/RIV, respectively. These were constructed by isolating the EcoRI–Eagl [5′–β] fragment from each corresponding p8A mutant and inserting it into the corresponding site of Acate30. Two DNA fragments from an EcoRI and Eagl digest of Acate30 were isolated: One contains the sequences from −10 of the β-globin promoter to +250 of the CAT gene (CAT′), and the second contains the remaining enhancer–vector sequences [Enh–vector]. The mutated p8A 5′–β′ and CAT′ fragments were then ligated together, digested with EcoRI, and ligated to the Enh–vector fragment. The resulting Acate derivatives, containing mutations in the −30 GATA box, were digested with Psrl to determine the orientation of the 5′–β′CAT′ fragment.

Preparation and purification of protein extracts

Nuclear protein extracts from adult and 11-day chick erythrocytes (McIntyre Poultry, San Diego, CA) were prepared as described previously (Emerson et al. 1987). High-affinity DNA-binding proteins from staged erythrocytes were obtained by chromatography of a 0.42 M KCl nuclear extract on calf thymus DNA–cellulose (Sigma) and eluting with 0.25 M [NH4]2SO4 (DC/250 fraction) as described in Emerson and Felsenfeld (1984). Purified cGATA-1 protein was prepared by chromatographing a DC/250 preparation on a sequence-specific GATA (enhancer) region IV DNA–affinity column according to the method of Kadonaga and Tian (1986).

DNase protection analyses and gel mobility shifts

DNase protection experiments were conducted as described by Emerson et al. (1987). The analysis of cGATA-1 and yeast or hTFIID interaction with 5′ 32P-labeled oligonucleotides to various TATA sequences was performed by incubation of protein and DNA in a buffer containing 20 mM HEPES (pH 7.9), 50 mM NaCl, 5 mM MgCl2, 100 μg/ml of BSA, 6% glycerol, 1 mM DTT, and 0.025% NP-40. Binding was allowed to proceed for 30 min at 30°C. The reactions were then analyzed by electrophoresis on...
a 5% polyacrylamide gel containing 0.5× TBE, 0.05% NP-40, 5% glycerol, 0.5 mM DTT, and 1 mM EDTA [pH 7.5]. Gels were run in 0.5× TBE and 1 mM EDTA [pH 7.5] for 1 hr at 250 V at 4°C.

Oligonucleotides used in these experiments were synthesized by Operon Technologies [Alameda, CA] and are as follows:

**GATA** 34-mer
5'-GATCCCGGGAGAAGCAGAGGCTGGGCGCTC-3' 5'-GGCCGGGTCGTCCTCCCGGATC-5' 5'-GATCCCGGGAGAAGCAGAGGCTGGGCGCTC-3' 5'-GGCCGGGTCGTCCTCCCGGATC-5' 5'-GATCCCGGGAGAAGCAGAGGCTGGGCGCTC-3' 5'-GGCCGGGTCGTCCTCCCGGATC-5' 5'-GATCCCGGGAGAAGCAGAGGCTGGGCGCTC-3' 5'-GGCCGGGTCGTCCTCCCGGATC-5'

**GGA** 34-mer
5'-GATCCCGGGAGAAGCAGAGGCTGGGCGCTC-3' 5'-GGCCGGGTCGTCCTCCCGGATC-5' 5'-GATCCCGGGAGAAGCAGAGGCTGGGCGCTC-3' 5'-GGCCGGGTCGTCCTCCCGGATC-5' 5'-GATCCCGGGAGAAGCAGAGGCTGGGCGCTC-3' 5'-GGCCGGGTCGTCCTCCCGGATC-5' 5'-GATCCCGGGAGAAGCAGAGGCTGGGCGCTC-3' 5'-GGCCGGGTCGTCCTCCCGGATC-5'

**SSE** 30-mer
5'-GATCCCGGGAGAAGCAGAGGCTGGGCGCTC-3' 5'-GGCCGGGTCGTCCTCCCGGATC-5' 5'-GATCCCGGGAGAAGCAGAGGCTGGGCGCTC-3' 5'-GGCCGGGTCGTCCTCCCGGATC-5' 5'-GATCCCGGGAGAAGCAGAGGCTGGGCGCTC-3' 5'-GGCCGGGTCGTCCTCCCGGATC-5' 5'-GATCCCGGGAGAAGCAGAGGCTGGGCGCTC-3' 5'-GGCCGGGTCGTCCTCCCGGATC-5'

**In vitro transcription**

Conditions for transcription and the preparation of extracts from 11-day chick erythrocytes were identical to that described previously (Emerson et al. 1989). The preparation of whole-cell transcription extracts from HeLa cells was according to a modification of the procedure of Dignam et al. (1983). Reaction products were analyzed by S1 nuclease digestion and electrophoresed on 8% polyacrylamide/urea/TBE gels. Transcription reactions were quantitated by excising the ^32P-labeled 94-base RNA fragment from isolated mammalian nuclei. Nucleic Acids Res. 11: 1475–1489.

**Transfections**

Primary erythrocytes isolated from the circulation of 10- to 11-day chick embryos [McIntyre Poultry] were osmotically shocked by treatment with 0.25 m NH4Cl and transfected with various β-globin DNA constructs. Definitive erythroid cells [3×10^6 to 5×10^6] were transfected with 3 μg/ml of DNA according to the method of Hesse et al. (1986), as modified by Jackson et al. (1989). Following a 48-hr incubation at 37°C, the cells were processed and assayed for CAT activity using a standard protocol based on the method of Gorman et al. (1982).

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**References**

Berger, S.L., W.D. Cress, A. Cress, S.J. Triezenberg, and L. Guarente. 1990. Selective inhibition of activated but not basal transcription by the acidic activation domain of VP-16: Evidence for transcriptional adaptors. Cell 61: 1199–1208.

Brown, J.L. and V.M. Ingram. 1974. Structural studies on chick embryonic hemoglobins. J. Biol. Chem. 249: 3960–3972.

Brus, G.A. and V.M. Ingram. 1973. The erythroid cells and hemoglobins of the chick embryo. Phil. Trans. R. Soc. Lond. B Biol. Sci. 266: 225–305.

Chapman, B.S. and A.J. Tobin. 1979. Distribution of developmentally regulated hemoglobins in embryonic erythroid populations. Dev. Biol. 69: 375–387.

Chen, W. and K. Struhl. 1988. Saturation mutagenesis of a yeast his3 “TATA element”: Genetic evidence for a specific TATA-binding protein. Proc. Natl. Acad. Sci. 85: 2691–2695.

Choi, O.B. and J.D. Engel. 1988. Developmental regulation of β-globin gene switching. Cell 55: 17–26.

Corbin, V. and T. Maniatis. 1989. The role of specific enhancer-promoter interactions in the Drosophila Adh promoter switch. Genes & Dev. 3: 2191–2200.

Dignam, J.D., R.M. Lebovitz, and R.G. Roeder. 1983. Accurate cloning of eukaryotic DNA fragments. Methods Enzymol. 100: 261–284.

Dynlacht, B.D., T. Hoey, and R. Tjian. 1991. Isolation of coactivators associated with the TATA-binding protein that mediate transcriptional activation. Cell 66: 563–576.

Emerson, B.M. and G. Felsenfeld. 1984. Specific factor conferring nuclear hypersensitivity at the 5' end of the chicken adult β-globin gene. Proc. Natl. Acad. Sci. 81: 95–99.

Emerson, B.M., C.D. Lewis, and G. Felsenfeld. 1985. Interaction of specific nuclear factors with the nuclear-hypersensitive region of the chicken adult β-globin gene: Nature of the binding domain. Cell 41: 21–30.

Evans, T. and G. Felsenfeld. 1989. The erythroid-specific transcription factor Eryf1: A new finger protein. Cell 58: 877–
Evans, T., M. Reitman, and G. Felsenfeld. 1988. An erythrocyte-specific DNA-binding factor recognizes a regulatory sequence common to all chicken globin genes. Proc. Natl. Acad. Sci. 85: 5976–5980.

Gallarda, J.L., K.P. Foley, Z. Yang, and J.D. Engel. 1989. The β-globin stage selector element factor is erythroid-specific promoter/enhancer binding protein NF-E4. Genes & Dev. 3: 1845–1859.

Gorman, C.M., I.F. Moffat, and B.H. Howard. 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. 2: 1044–1051.

Harbury, P.A.B. and K. Struhl. 1989. Functional distinctions between yeast TATA elements. Mol. Cell. Biol. 9: 5298–5304.

Hesse, J.E., J.M. Nickol, M.R. Lieber, and G. Felsenfeld. 1986. Regulated gene expression in transfected primary chicken erythrocytes. Proc. Natl. Acad. Sci. 83: 4312–4316.

Ho, I.C., L.-H. Yang, G. Morle, and J.M. Leiden. 1989. A T cell specific transcriptional enhancer element 3′ of Coα in the human T cell receptor α locus. Proc. Natl. Acad. Sci. 86: 6714–6718.

Ho, I.C., P. Vorhees, N. Marin, B.K. Oakley, S.-F. Tsai, S.H. Orkin, and J.M. Leiden. 1991. Human GATA-3: A lineage-restricted transcription factor that regulates the expression of the T cell receptor α gene. EMBO J. 10: 1187–1192.

Horikoshi, N., K. Maguire, A.R. Kralli, E. Maldonado, D. Reinberg, and R. Weinmann. 1991. Direct interaction between adenovirus E1A protein and the TATA box binding transcription factor IID. Proc. Natl. Acad. Sci. 88: 5124–5128.

Jackson, P.D., T. Evans, J.M. Nickol, and G. Felsenfeld. 1989. Developmental modulation of protein binding to β-globin gene regulatory sites within chicken erythrocyte nuclei. Genes & Dev. 3: 1860–1873.

Kadonaga, J.T. and R. Tjian. 1986. Affinity purification of sequence-specific DNA binding proteins. Proc. Natl. Acad. Sci. 83: 5889–5893.

Kelleher, R.J. III, P.M. Flanagan, and R.D. Kornberg. 1990. A novel mediator between activator proteins and the RNA polymerase II transcription apparatus. Cell 61: 1209–1215.

Ko, L.J., M. Yamamoto, M.W. Leonard, K.M. George, P. Ting, and J.D. Engel. 1991. Murnane and human T lymphocyte GATA-3 factor mediate transcription through a cis regula-tory element within the human T-cell receptor δ gene enhancer. Mol. Cell. Biol. 11: 2778–2784.

Lee, W.S., C.C. Kao, G.O. Bryant, X. Liu, and A.J. Berk. 1991. Adenovirus E1A activation domain binds the basic repeat in the TATA box transcription factor. Cell 67: 365–376.

Lewis, C.D., S.P. Clark, G. Felsenfeld, and H. Gould. 1988. An erythrocYTE-specific protein that binds to the poly[dG] region of the chicken β-globin gene promoter. Genes & Dev. 2: 863–873.

McCormick, A., H. Brady, J. Fukushima, and M. Karin. 1991. The pituitary-specific regulatory gene GHFI contains a minimal cell type-specific promoter centered around its TATA box. Genes & Dev. 5: 1490–1503.

McGhee, J.D., W.I. Wood, M. Dolan, J.D. Engel, and G. Felsenfeld. 1981. A 200 base pair region at the 5′ end of the chicken adult β-globin gene is accessible to nuclease digestion. Cell 27: 45–55.

Meisterernst, M., A.L. Roy, H.M. Lieu, and R.G. Roeder. 1991. Activation of class II gene transcription by regulatory factors is potentiated by a novel activity. Cell 66: 981–993.

Nakajima, N., M. Horikoshi, and R.G. Roeder. 1988. Factors involved in specific transcription by mammalian RNA polymerase II: purification, genetic specificity, and TATA box-promoter interactions of TFII D. Mol. Cell. Biol. 8: 4028–4040.

Nickol, J.M. and G. Felsenfeld. 1988. Bidirectional control of the chicken β- and ε-globin genes by a shared enhancer. Proc. Natl. Acad. Sci. 85: 2548–2552.

Pevny, L., M.C. Simon, E. Robertson, W.H. Klein, S.-F. Tsai, V. D’Agati, S.H. Orkin, and F. Costantini. 1991. Erythroid differentiation in chimaeric mice blocked by a targeted mutation in the gene for transcription factor GATA-1. Nature 349: 257–260.

Plumb, M.A., V.V. Lobanenkov, R.H. Nicolas, C.A. Wright, S. Zavou, and G.H. Goodwin. 1986. Characterisation of chicken erythroid nuclear proteins which bind to the nuclelease hypersensitive regions upstream of the adult β- and hatching β-globin genes. Nucleic Acids Res. 14: 7675–7693.

Plumb, M.A., J. Frampton, H. Wainwright, M. Walker, K. Macleod, G. Goodwin, and P. Harrison. 1989. GATAA gene, a cis-control region binding an erythroid-specific nuclear factor with a role in globin and nonglobin gene expression. Nucleic Acids Res. 17: 73–91.
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**References**

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