The Interaction of GATA-binding Proteins and Basal Transcription Factors with GATA Box-containing Core Promoters

A MODEL OF TISSUE-SPECIFIC GENE EXPRESSION*

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The core promoters of the rat platelet factor 4 (PF4), mouse erythropoietin and chicken β globin genes contain a GATA motif in place of the consensus TATAAA site. In the case of the PF4 gene, this site has been shown to play a critical role in restricting transcription to the megakaryocyte lineage. In order to understand the mechanism of tissue specificity, we investigated the function of the GATA box-containing promoters in vitro.

Our studies show that the TATA-binding protein of TFIIID is required for initiation of transcription from the GATA box-containing promoters. GATA-1 interacts with the core promoter GATA motif and inhibits generation of preinitiation complexes. The functional significance of the inhibition of preinitiation complexes is supported by in vitro transcription assays in which transcription from the PF4 and erythropoietin core promoters is suppressed by GATA-1. We also demonstrate that GATA-2 inhibits initiation of transcription from the PF4 core promoter. Based on these results, we propose a model in which repression of PF4 expression in nonmegakaryocytes is mediated, in part, by competition between GATA-binding proteins and basal factors for the core promoter.

Pluripotent bone marrow stem cells give rise to the diploid megakaryoblast, which subsequently matures into the polyploid megakaryocyte. The critical events that govern differentiation of the stem cell to the megakaryocyte are unknown. The delineation of molecular interactions involved in megakaryocyte-specific gene expression should provide a framework for defining this pathway. The 1.1-kilobase upstream region of the rat platelet factor four (PF4) promoter coupled to the β-galactosidase gene has been employed to produce several lines of transgenic mice. Marker enzyme production in the bone marrow is limited to megakaryocytes, suggesting that this region contains the tissue-specific regulatory elements (Ravid et al., 1991a). In transient transfection assays using rat bone marrow, deletion analysis of the PF4 promoter has led to the identification of 5' upstream sequences that regulate transcription in the megakaryocyte and inhibit expression in other hematopoietic cell types (Ravid et al., 1991b). Surprisingly, a single point mutation of a GATA motif (−31 to −28) to the canonical TATA sequence leads to low level expression in non-megakaryocyte cells (Ravid et al., 1991b). In view of the critical role of this motif in directing tissue-specific expression, we asked how initiation of transcription from the PF4 promoter differs from TATA-containing promoters.

Initiation of transcription from all class II promoters is believed to be mediated by the assembly of a common set of general factors on the core promoter. Usually, this process begins with the binding of TFIIID to the consensus TATAAA and is followed by the recruitment of other general factors, leading to the formation of a multi-protein initiation complex (Sawadogo and Roeder, 1985; Buratowski et al., 1989). The TATAAA consensus sequence is not only important as a recognition site for TFIIID, it also plays a role in defining an accurate start site and determining the direction of transcription. The sequence of events leading to initiation of transcription from the minority of promoters that lack a defined TATA box is less clear. In some cases, TFIIID may bind specifically to the −30 site, despite the absence of the consensus TATAAA (Hahn et al., 1989; Wiley et al., 1992). In other instances, an initiator element may play an important role in localizing the start of transcription (Smale and Baltimore, 1989). In TATA-less promoters that contain an upstream SPI-binding site, TFIIID appears to be associated with the promoter via protein-protein interactions involving a tethering factor (Pugh and Tjian, 1989). Thus, the above data suggest that the only difference between promoters that contain a TATA box and those that lack this sequence is the mechanism by which TFIIID is recruited to the preinitiation complex.

The rat PF4 core promoter, as well as the mouse erythropoietin and chicken β globin promoters not only lack the consensus TATAAA sequence, but in its place contain a GATA motif. The GATA element was initially identified as an upstream cis regulatory site in erythroid, megakaryocyte, and mast cell linesages (Tsai et al., 1989; Evans and Felsenfeld, 1989). Subsequent studies have led to the isolation of related proteins (GATA-2 to GATA-4) that share a highly conserved zinc finger DNA-binding domain but differ widely in tissue distribution (Yamamoto et al., 1990; Lee et al., 1991; Ho et al., 1991; Arceci et al., 1993). GATA factors bind to

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† The abbreviations used are: PF4, platelet factor 4; TF, transcription factor; TBP, TATA-binding protein; bp, base pairs; MLP, major late promoter.
In vitro transcription templates were constructed by inserting the above genes and were constructed with double-stranded oligonucleotides containing a 5'-terminal XhoI site linked to -50 to +3 of the β-globin gene promoter. These fragments were inserted into the EcoRI and Smal sites of pUC19 and the resultant constructs were used to generate probes for electrophoretic mobility shift assays. As a group, these plasmids were designated p(core)PUC19. In vitro transcription templates were constructed by inserting the above promoter fragments between the EcoRI and blunt-ended SacI sites of the G-less cassette template (Sawadogo and Roeder, 1985). The construction of pALM200I has been described elsewhere (Buratowski et al., 1988). To obtain the GATA-1-expressing plasmid pETHisGATA-1, the cDNA-containing XhoI fragment of PXMcGATA (Martin and Orkin, 1990) was blunt ended and inserted into the blunt-ended Ncol site of PET15b (Novagen). HeLa S8 (ATCC CCL 2.2) and HEL (ATCC TIB 180) cells were grown, and nuclear extracts were prepared as previously described (Dignam et al., 1983; Shapiro et al., 1988).

Purification of Basal Transcription Factors and GATA Protein—Recombinant mouse GATA-1 was expressed in Escherichia coli (BL21(DE3)) carrying the plasmid pETHisGATA-1. An overnight 5-ml culture was diluted into 500 ml of LB medium with ampicillin and grown at 37 °C. When the A600 reached 0.5–0.7, 0.5 ml isopropyl-1-thio-β-D-galactopyranoside was added and the culture was incubated for an additional 2 h. The bacteria were then centrifuged and the pellet was resuspended in 10 ml of 10 mM Tris-HCl (pH 8.0), 0.5% Nonidet P-40, 2 mM PMSF, and 0.1 mM NaF. Triton X-100 (0.2% final) was added, and the cells were sonicated and then incubated at room temperature for 1 h. Following centrifugation, the supernatant was passed through a 45-μm filter and then applied to a 2-ml nickel-NTA column that was pre-equilibrated with binding buffer. The column was washed with 10 ml of binding buffer (pH 7.4). Frac- tions containing GATA-1 were identified by their ability to specifically bind to a probe containing a GATA motif in a mobility shift assay. Western blot assays employing SDS-polyacrylamide gel electrophoresis revealed two GATA-1 bands (48 and 43 kDa), which were responsible for greater than 50% of the GATA-containing protein. Recombinant GATA-1 was reatured by dialyzing the fractions against buffer D (20 mM Hepes (pH 7.9), 0.1% Nonidet P-40, 0.5 mM phenylmethylsulfonyl fluoride, 2 mM dithiothreitol, 300 mM KCl, 10% glycerol, 100 μM ZnSO4) containing decreasing amounts of urea. The final dialysis was against binding buffer D without urea and with 100 mM KCl. Histidine-tagged human TBP was expressed in E. coli and isolated as described (Dignam et al., 1983; Shapiro et al., 1988). Human TFIIB was expressed in a baculovirus expression system (Parvin et al., 1992). HeLa cell fraction containing TFIIF (TFIIH), RNA polymerase II, and the 70-kDa transcription modulator. When transcriptions were performed in the absence of GTP, the internal control pALM200I template generated RNA transcripts of 210 nucleotides, and the test templates generated RNA products of 382 nucleotides. Each reaction contained 100 ng each of test and control supercoiled plasmid DNA, 0.1 mM ATP, 0.1 mM GTP, 0.003 mM CTP, 0.05 mM 3'-Omethylated GTP, 15 units of RNasin (Promega Biotech), 10 μCi of [32P]CTP (800 Ci/mmol, Du Pont NEN), 20 mM Hepes (pH 7.9), 1 mM EDTA, 5 mM MgCl2, 60 mM KCl, 12% glycerol, and 0.15 mg/ml bovine serum albumin. A typical reaction contained 20–40 ng of TBP, 35 ng of recombinant TFIIB, 0.5 μl of TFIIA fraction, 1.5 μl of TFIIF(E/H) fraction, 4 ng of recombinant TFIE, 0.5 μl of RNA polymerase II fraction, and 3 μl of 700-kDa protein fraction. Reactions (25 μl) were assembled on ice and then incubated at 30 °C for 30 min. The DNA-protein complexes were separated by electrophoresis on a 4% nondenaturing polyacrylamide gel containing 25 μM Tris, 200 mM glycine, 1 mM EDTA.

In Vitro Transcription Reactions—Transcription reactions using partially purified basal factors were performed in 25-μl reactions using 10 units of TFIIE(E/H), RNA polymerase II, and the 700-kDa transcription modulator. When transcriptions were performed in the absence of GTP, the internal control pALM200I template generated RNA transcripts of 210 nucleotides, and the test templates generated RNA products of 382 nucleotides. Each reaction contained 100 ng each of test and control supercoiled plasmid DNA, 0.1 mM ATP, 0.1 mM GTP, 0.003 mM CTP, 0.05 mM 3'-Omethylated GTP, 15 units of RNasin (Promega Biotech), 10 μCi of [32P]CTP (800 Ci/mmol, Du Pont NEN), 20 mM Hepes (pH 7.9), 1 mM EDTA, 5 mM MgCl2, 60 mM KCl, 12% glycerol, and 0.15 mg/ml bovine serum albumin. A typical reaction contained 20–40 ng of TBP, 35 ng of recombinant TFIIB, 0.5 μl of TFIIA fraction, 1.5 μl of TFIIF(E/H) fraction, 4 ng of recombinant TFIE, 0.5 μl of RNA polymerase II fraction, and 3 μl of 700-kDa protein fraction. Reactions (25 μl) were assembled on ice and then incubated at 30 °C for 60 min and terminated by the addition of 0.15 ml of 0.36 M sodium acetate, 10 mM EDTA, 0.05% SDS, and 12.0 mg/ml tRNA. RNA products were then phenol-extracted, chloroform-extracted, precipitated in ethanol, and subjected to electrophoresis on 8% polyacrylamide in the presence of 8 M urea.

Transcription products were also tested for DNA-protein complexes using a phoshorimager (Molecular Dynamics, Sunnyvale, CA) with ImageQuant 2.0 software.

RESULTS

Basal Factors Bind to the GATA Box and Are Inhibited by GATA-1—The binding of highly purified proteins to the core promoter templates was examined in an electrophoretic mobility shift assay. The formation of complexes of the PF4 probe with the TATA-binding protein subunit of TFID and TFIIA resulted in a distinct DA complex (Fig. 1b, lane 2). Inclusion of TFIIA, RNA polymerase II, and TFIIF(E/H) in the reaction mixture resulted in a DAB complex and a slowly migrating DABPoIF preinitiation complex (Fig. 1b, lane 3). Slower migrating TFIIE- and TFIIF-containing complexes were not observed under these conditions. The basal factor complexes did not form when TBP was omitted from the reaction mix (data not shown). Incubation of the PF4 probe with GATA-1 resulted in two distinct complexes (Fig. 1b, lane 4). These complexes were specifically competed by inclusion in the binding reactions of a 100-fold molar excess of unlabeled probe containing a GATA motif but not by a similar concentration of probe in which the GATA site was mutated to TATA (Fig. 1c, lanes 2 and 3). Incubation of erythropoietin and β globin probes with basal factors or with GATA-1 also resulted in specific DNA-protein complexes, which were isolated and identified as described previously (Ha et al., 1991). Histidine-tagged human TBP was expressed in bacteria and purified by nickel-NTA chromatography (Parvin et al., 1992). The TBP preparation was approximately 10% pure as estimated by Coomassie-stained SDS gels. The subunits of TFIIIE were expressed and purified as outlined previously (Parvin et al., 1992). A HeLa cell fraction containing TFIIF, TFIIH, and TFIIE (referred to as TFIIF(E/H)) was purified by phosphocellulose and DEAE-Sepharose chromatography, followed by gel filtration as described in a prior communication (Parvin et al., 1992). Chinese hamster ovary cell amantidin-resistant RNA polymerase II was prepared as previously outlined (Carthew et al., 1988). The modulatory 700-kDa activity was a HeLa cell fraction purified by phosphocellulose and DEAE-Sepharose chromatography and gel filtration (Parvin et al., 1992).
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**FIG. 1. Basal factors and GATA-1 bind to the GATA box-containing promoters.** Panel a, the DNA sequences of the rat PF4, rat PF4(T-31), mouse erythropoietin, and chicken β globin gene core promoters are provided. The transcriptional start sites are indicated by an arrow, and the GATA or TATA motifs are underlined. These promoter sequences are directly ligated to the 380-bp G-less cassette sequence. Panel b, the binding reactions employed 0.2 ng of probe (PF4, lanes 1–4; PF4(T-31), lanes 5–7; erythropoietin, lanes 8–10; β globin, lanes 11–13). The proteins added to the reaction mix were 50 ng of TBP, 1.0 pl of TFIIA fraction (lanes 2, 3, 5, 6, 8, 9, 11, and 12), 70 ng of TFIIB fraction, 1 pl of RNA polymerase II, and 1 pl of TFIIIE/H) fraction (lanes 3, 6, 9, and 12), and 250 ng GATA-1 (lanes 4, 7, 10, and 13). The protein labeled IF represents the TFIIIE/H) fraction. The GATA-1 complex is indicated by a double arrow. The basal factor complexes are labeled DA (TBP, TFIIA); DAB (TBP, TFIIA, TFIIB); DABP(1F (TBP, TFIIA, TFIIH, RNA polymerase II, TFIIE). Panel c, the binding reactions included 0.2 ng of PF4 probe and 250 ng of GATA-1 in the absence or presence of 100-fold molar excess of cold probe representing PF4 (lane 2) or PF4(T-31) (lane 3).
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**Figure 2.** GATA-1 competes with binding of basal factors to the GATA box-containing promoters. All binding reactions employed core promoter probe and TBP, TFIIA, TFIIIB, RNA polymerase II, as well as TFIIIE/F/H at the same concentrations as in Fig. 1. The binding reactions also utilized GATA-1 at the same levels as in Fig. 1 (lanes 2, 4, 6, and 8). All proteins were added simultaneously to the reaction mix. The protein-DNA complexes are labeled as in Fig. 1. A slowly migrating complex is indicated with an asterisk.

GATA-1 could disrupt a preformed preinitiation complex. When TBP, TFIIA, and GATA-1 were added to the reaction mix together, GATA-1 competed for binding with the DA complex (Fig. 3, lane 2). When the probe was incubated with TBP and TFIIA alone, subsequent addition of GATA-1 also resulted in inhibition of the DA complex (Fig. 3, lane 3). However, on dark exposure of this autoradiogram, the preformed DA complex was competed less efficiently (data not shown). Similarly, late addition of GATA-1 to a reaction mixture that contained TBP, TFIIA, TFIIIB, RNA polymerase II, and TFIIIE/F/H fraction also resulted in less inhibition of the DAB and DABPolF complexes (Fig. 3, lane 6).

The Basal Factors Have Lower Affinity for the GATA Box Compared with the TATA Box—the relative affinity of TBP for GATA box-containing promoters was determined by incubating a radiolabeled probe containing the adenovirus major late promoter (MLP) core promoter with TBP and TFIIA in the presence or absence of excess unlabeled GATA box-containing probe. The MLP contains a TATAAA consensus sequence that efficiently binds the DA complex (Buratowski et al., 1989) (Fig. 4, lane 1). The addition of excess unlabeled core promoter DNA to the reaction resulted in varying degrees of competition of the DA complex (Fig. 4, lanes 2–16). In reactions that contained a 200 molar excess of cold probe (Fig. 4, lanes 2, 5, 8, 11, and 14), the activity of the DA complex relative to the control (Fig. 4, lane 1) was as follows: MLP, 2%; PF4, 1.3%; β globin, 17%; PF4, 30%; erythropoietin, 84%. These values provide an estimate of the relative affinity of the DA complex for the promoters: MLP > PF4 > β globin > PF4 > erythropoietin. A similar spectrum of affinities of the DAB and DABPolF complexes for the core promoters was observed (data not shown).
core promoter complex was competed by varying concentrations of unlabeled GATA box-containing probe, the relative affinity of GATA-1 for the GATA motif was erythropoietin > PF4 > β globin (data not shown).

Initiation of Transcription from PF4 and Erythropoietin Is Inhibited by GATA-1 and GATA-2—In order to determine the functional significance of the protein interactions described above, we measured the in vitro transcriptional activity of GATA box-containing promoters in the presence or absence of GATA protein. Recombinant GATA-1 protein has been shown to stimulate in vitro transcription from templates that contain upstream GATA motifs (Kim et al., 1990). Transcription from PF4, erythropoietin, and β globin was reconstituted with basal factors provided either as HeLa nuclear extract (Fig. 5, lanes 1–4), nuclear extract from a human erythroleukemia cell line (HEL) (Fig. 5, lanes 5–8), or as a set of highly purified proteins known to be sufficient for initiation of transcription from TATA-containing promoters (Fig. 7, lanes 1–4). HeLa cells are known to contain GATA-2, while HEL cells contain both GATA-1 and GATA-2. The basal level of transcription from GATA box-containing promoters was lower as a group when compared to PF4T-31. Within the group, there was a spectrum of activity with β globin > PF4 > erythropoietin. These differences were consistent whether the source of basal factors was HeLa cell, HEL cell, or purified proteins, suggesting that a common set of basal factors is sufficient for initiation of transcription from the GATA box-containing promoters. As expected, transcription did not occur with the PF4 promoter when TBP was omitted from the reaction mix (Fig. 6, lanes 1 and 3). The importance of TBP in transcriptional initiation is further supported by the observation that activity in vitro is correlated with the relative affinity of the DA complex for the promoter (compare Figs. 5 and 4). Finally, GATA-1 is not able to replace TBP in the initiation of transcription (Fig. 6, lane 3).

In the presence of GATA-1, transcription from wild-type and mutant PF4T-31 promoters is partially suppressed in reactions containing TBP or TFIIID. In multiple experiments using different preparations of GATA-1, the magnitude of inhibition of transcription from the GATA-containing PF4 promoter was 2–4-fold greater than the extent of suppression of transcription from the PF4T-31 promoter in reactions containing TBP (Fig. 7, lanes 1, 2, 5, and 6). A similar phenomenon was observed with the erythropoietin promoter as compared to the TATA box-containing MLP (Fig. 7, lanes 3 and 7). Prolonged exposure of the autoradiograph showed that transcription from the erythropoietin promoter in the presence of GATA-1 was undetectable (data not shown). In contrast, transcription from the β globin promoter was minimally inhibited by addition of GATA-1 to the reaction mix (Fig. 7, lanes 4 and 8). These differences in the extent of competition appear to be due to the degree of inhibition of basal factor complex formation by GATA-1 (see Fig. 2), which is consistent with the relative affinities of GATA-1 and basal factors for the core promoters (see Fig. 4). Thus, GATA-1 mainly inhibits initiation of transcription from GATA box-containing promoters by steric hindrance of basal factor complex formation but to a lesser extent also suppresses DABPolF preinitiation complex formation via protein-protein interactions. It is of interest that GATA-2 seems to exhibit less template-nonspecific inhibition of transcription. The addition of GATA-2 to a reconstituted transcription reaction produces significant suppression of transcription from the PF4 promoter (Fig. 8, lanes 1 and 2) but essentially no change in transcription from PF4T-31 (Fig. 8, lanes 3 and 4). These variations in the inhibitory effects of GATA-1 and GATA-2 are due either to the different structures of the two proteins or the use of a slightly truncated form of GATA-2. Finally, in transcription reactions containing TFIIID instead of TBP, the addition of GATA-1 resulted in strong inhibition from all test templates, while addition of GATA-2 resulted in specific inhibition of transcription from PF4 (data not shown).

**DISCUSSION**

In the present study, we demonstrate that GATA-1 competes with basal factors for binding to the PF4, erythropoietin, and β
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![Diagram](image)

**Fig. 6.** TBP is required for initiation of transcription from PF4 and cannot be replaced by GATA-1. The transcription reactions employed 100 ng of PF4 and MLP templates, as well as 0.5 pl of TFIIB fraction, 70 ng of TFIIA fraction, 0.5 pl of RNA polymerase II fraction, 4 ng of TFIIH, 1.5 pl of TFIIF (E/H) fraction, 56 ng of TBP (lanes 2 and 4), or 200 ng of GATA-1 (lanes 3 and 4). The transcripts intermediate in size between the test and MLP transcripts represent nonspecific internal initiations or premature termination of transcription from the test template.

![Diagram](image)

**Fig. 7.** Initiation of transcription from PF4 and erythropoietin is inhibited by GATA-1. The transcription reactions employed 100 ng of test template (PF4, lanes 1 and 5; PF4poly, lanes 2 and 6; erythropoietin, lanes 3 and 7; β globin, lanes 4 and 8), 100 ng MLP, and TBP, TFIIA, TFIIIB, PolII, TFIIH, TFIIF, and TFIIE at concentrations provided in Fig. 6, as well as 3 pl of the 700-kDa activity. Also included in the reaction mixtures was 200 ng of GATA-1 (lanes 5–8). The RNA transcripts are labeled as in Fig. 5.

![Diagram](image)

**Fig. 8.** Initiation of transcription from PF4 is inhibited by GATA-2. The transcription reactions employed 100 ng of test template (PF4, lanes 1 and 2; PF4poly, lanes 3 and 4), 100 ng MLP, and basal factors as described in Fig. 7. Approximately 100 ng of GATA-2 was included in the reaction mixtures (lanes 2 and 4).

**Note:** In a mobility shift assay, Fong and Emerson (1992) showed that the addition of a calf thymus DNA flow-through fraction of erythroid nuclear extract resulted in a supershift of the TBP-β globin promoter complex. Since the supershifted complex was competed poorly by GATA-1 compared with the TBP-promoter complex, the authors speculated that the flow-through fraction contained an "adaptor" that promoted preferential binding of TBP to the presence of GATA-1 (Fong and Emerson, 1992). We repeated these experiments with HeLa cell extract and found that addition of the calf thymus DNA flow-through fraction to binding reactions resulted in a supershift of TBP-β globin promoter that comigrated with the DA complex. Subsequent addition of TFIIB to the reaction mix resulted in a more slowly migrating complex that comigrated with the DAB complex (data not shown). Finally, formation of the TBP-adaptor complex was not dependent on the presence of a GATA motif; a similar supershift formed on promoters that contained a TATA box, but it was not dependent on the presence of a GATA motif; a similar supershift formed on promoters that contained a TATA box, but it was not dependent on the presence of a GATA motif.

Steric interference of preinitiation complex formation has been described for other proteins, including the Engrailed homodomain, the Drosophila P-element transposase, as well as the BPV-1 E2-transactivating proteins, and may constitute a general mechanism of negative gene regulation (Distelhaur, 1991; Okuma et al., 1991; Kaufman and Rio, 1991). In this case, the effect is mediated by specific transcription factors that have a dual function depending on the location of its cognate site relative to the TRP-binding site. In upstream promoter regions these factors function as transcriptional activators while in the vicinity of the TATA box, they inhibit initiation of transcription by interfering with the assembly of basal factor complexes. This is the first study to identify GATA-binding proteins as members of this group of transcriptional repressors. The minimum distance between the GATA motif and the TBP-binding site that determines whether GATA-1 functions as an activator or repressor of transcription has not been established. However, we note that the TATA-less glycophorin B promoter, which contains a GATA motif at −40, is activated by GATA-1 in a cotransfection assay (Rahuel et al., 1992). Based upon our results, we suggest that the above GATA motif must be sufficiently upstream of the TBP-binding site to preclude steric interference of preinitiation complex formation.

Hematopoietic bone marrow cells, which normally express PF4 and β globin, also synthesize GATA-binding proteins. The mechanism by which these cells circumvent the inhibitory effect of GATA factors has not been established. It has been postulated that an adaptor protein mediates preferential binding of basal factors to the chicken β globin promoter, allowing for appropriate expression in a GATA-1-containing environment (Fong and Emerson, 1992). However, the proposed adaptor protein appears to be TFIIA.2 We believe that formation of repression may have biologic importance. For example, in transient transfections using rat bone marrow, a single point mutation of G-31 to the consensus TATAAA in the PF4 promoter results in expression in the non-megakaryocytic cells (Ravid et al., 1991b). If the increased transcription in these cell types was due merely to the introduction of a consensus TATA motif, the mutation should also have resulted in higher levels of expression in megakaryocytes. Since this was not observed (Ravid et al., 1991b), we believe the results to be consistent with an inhibitory effect of the binding of a GATA factor to the −31 GATA site. It is difficult to associate this biologic effect with a single transacting factor since more than one GATA binding protein binds to the same consensus GATA motif in vitro (Dorfman et al., 1992). However, given the limited expression of GATA-1, we suggest that a more widely distributed GATA protein such as GATA-2 may be responsible for transcriptional repression of PF4. In support of this possibility, we show that GATA-2 also inhibits initiation of transcription from PF4 in vitro.
preinitiation complexes in the presence of GATA binding proteins is more likely to be mediated by interaction of basal factors with tissue-specific transcription factors bound to upstream promoter sequences. It is noteworthy that the PF4 promoter contains a GATA-binding site at -134 (data not shown). Deletion of this region results in reduced expression of a reporter gene in megakaryocytes, suggesting that the upstream GATA motif acts as a positive regulatory element (Ravid et al., 1991b). An exciting possibility is that GATA-binding proteins serve to positively and negatively regulate PF4 expression at different stages of megakaryocyte development. In early progenitors cells, for example, GATA factor may bind to the -31 GATA site and repress PF4 gene expression. During later stages of development, GATA protein or a co-factor may bind to upstream sites, resulting in derepression and activation of transcription.

We have provided evidence that GATA box-containing core promoters exhibit a lower basal level of in vitro transcription as compared to those with a consensus TATAAA site. It is not surprising that the GATA(A) motif functions as a weak TATA box, since most point mutations of the consensus TATAAA site lead to reduced in vitro activity (Wobbe and Struhl, 1989, Chen and Struhl, 1988). However, in transient transfection assays, a point mutation in the chicken β globin promoter of GATA box (Ravid et al., 1988) to the GAL4 enhancer is comparable to wild type (Harbury and Struhl, 1989; Wobbe and Struhl, 1990). Together, these findings indicate that weak in vitro activity of certain TATA-less core promoters, including those with a GATA motif, may be compensated by upstream promoter sequences.

In summary, we have demonstrated that the GATA box is a recognition site for basal factors and GATA-binding proteins. The GATA-binding proteins inhibit transcription from the PF4 and erythropoietin promoters by interfering with formation of preinitiation complexes and may represent an important mechanism for repressing transcription in cell types that do not express these genes. The initiation of transcription in cells that normally express these genes as well as GATA-binding proteins is likely to occur via preferential binding of basal factors to the core promoter secondary to interaction with tissue-specific transcription factors bound to upstream promoter sequences.

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