Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 10444–10448, November 1992
Biochemistry

Functional erythroid promoters created by interaction of the transcription factor GATA-1 with CACCC and AP-1/NFE-2 elements

(globin gene regulation/hereditary persistence of fetal hemoglobin)

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Communicated by E. Donnall Thomas, July 9, 1992

ABSTRACT **We have investigated interactions between the erythroid transcription factor GATA-1 and factors binding two cis-acting elements commonly linked to GATA sites in erythroid control elements. GATA-1 is present at all stages of erythroid differentiation, is necessary for erythropoiesis, and binds sites in all erythroid control elements. However, minimal promoters containing GATA-1 sites are inactive when tested in erythroid cells. Based on this observation, two erythroid cis elements, here termed CACCC and AP-1/NFE-2, were linked to GATA sites in minimal promoters. None of the elements linked only to a TATA box created an active promoter, but GATA sites linked to either CACCC or AP-1/NFE-2 elements formed strong erythroid promoters. A mutation of T to C at position −175 in the γ-globin promoter GATA site, associated with hereditary persistence of fetal hemoglobin (HPFH), increased expression of these promoters in both fetal and adult cells. A construct bearing the β-globin CACCC element was more active in adult and less active in fetal erythroid cells, when compared with the γ-globin CACCC element. These studies suggest that erythroid control elements are formed by the interactions of at least three transcription factors, none of which functions alone.**

The genes of the human β-globin locus exhibit a tightly controlled pattern of regulation that permits very high levels of expression of each of the genes during particular stages of development (reviewed in refs. 1–4). They are arranged from 5′ to 3′ in order of their expression during development, when programmed switches in globin gene expression occur, from ε to γ during the embryonic period and from γ to β in the late fetal period (2–4). The individual globin promoters contain sufficient information for determining the developmental stages at which they are active, but are unable to drive the high level of expression seen in the context of the entire β-globin locus (5). High-level expression of globin genes requires the locus control region (LCR), an upstream region that enhances globin gene transcription and insulates the locus from the influence of flanking elements (6–9). Evidence from chickens and transgenic mice suggests that switching of the globin genes involves competition between globin promoters for enhancement by the LCR (10–12). The DNA sequences involved in controlling the level and stage of globin transcription, and the factors binding those sequences, have been intensively sought. While much information on the control of transcription level has been produced, the protein factors influencing the stage of globin expression remain largely unknown, and only GATA-1 has clearly been shown to be an erythroid transcription factor (13–15).

GATA-1, a zinc finger protein, is expressed in vertebrate erythroid cells and in the related megakaryocyte and mast cell lineages (16, 17). Its function in nonerythroid lineages is not well understood. Cis-acting DNA elements containing the core sequence motif GATA are bound by GATA-1; many of these elements have been characterized as sites of positive regulation in erythroid promoters and enhancers (13–15). GATA-1 is expressed at all stages of, and is necessary for, erythroid differentiation (1, 18). Its precise role in erythroid differentiation and gene regulation remains unclear. Cotransfection studies in nonerythroid cells established that GATA-1 could activate promoters containing only GATA sites linked to a TATA box and mapped an acidic activation domain at the amino terminus (19, 20). However, differences in the number and type of GATA site did not affect the activity of the minimal promoters. Furthermore, in erythroid cells, which express GATA-1 at high levels, these same minimal promoter constructs are inactive. A mutation in the GATA site from the γ-globin promoter (−175 T → C) is associated with hereditary persistence of fetal hemoglobin (HPFH), a condition in which γ-globin expression persists in cells normally expressing only β-globin (15, 21, 22). The HPFH promoter is 4–5 times more active than the wild-type promoter when tested in erythroleukemia cells; this alteration of promoter activity requires GATA-1 binding (15). However, when minimal promoters containing the wild-type and HPFH GATA elements from the $^{A}\gamma$-globin promoter were cotransfected into nonerythroid cells with GATA-1, there was no difference in promoter strength (19).

These results suggest that GATA-1 requires the presence of other factors to function in erythroid cells and that these may not be present in nonerythroid cells. These are presumably factors that bind near GATA sites in erythroid control elements. While numerous factors have been described as binding to globin promoters, none other than GATA-1 has been fully characterized. Studies of globin promoter function have found TATA, CCAAT, and CACCC sequences to be important in addition to GATA sites (22–25). In the core hypersensitive sites of the LCR, only GATA, CACCC, and AP-1/NFE-2 sites are protected when analyzed by *in vivo* footprinting (26, 47). Thus, we have speculated that GATA-1 interacts with factors binding to the CACCC and/or AP-1/NFE-2 elements.

CACCC elements are found close to GATA-1 sites in erythroid control elements (27). The factor(s) acting on these elements in erythroid cells has never been clearly characterized. Sp1, TEF-2, and at least two erythroid factors will bind CACCC elements, but which, if any, of these regulates the globin genes is unknown (28–30). Mutation of CACCC elements in the globin promoters sharply decreases promoter

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Abbreviations: LCR, locus control region; HPFH, hereditary persistence of fetal hemoglobin; hGH, human growth hormone; HEL, human erythroleukemia; MEL, mouse erythroleukemia; 5′HS2, 5′ hypersensitive site 2.

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strength (23, 24). Interaction of GATA and CACCC elements has been implied by the observation that activity of the porphobilinogen deaminase gene (*PBGD*) promoter is lowered when the spacing between GATA-1 and CACCC elements is increased (31).

The AP-1/NFE-2 element is found in the core hypersensitive sites of the LCR and in some erythroid promoters, but not in the globin promoters (32–35). It has been shown to be necessary for the powerful enhancing effect of 5′ hypersensitive site 2 (5′HS2) of the LCR and for activity of the *PBGD* promoter (36, 37). Again, no factor has been shown to be responsible for these effects; there is reason to suppose that more than one is involved. The elements are bound by AP-1, as well as an erythroid factor, NFE-2 (33, 34). AP-1 may interact with members of the hormone receptor family in complex ways to cause either positive or negative regulation (38, 39). The AP-1/NFE-2 element in the erythroid carbonic anhydrase II promoter has been shown to be regulated by the thyroid hormone receptor, c-ErbA, and its mutant counterpart v-ErbA (35). Thus, at least two generally expressed factors may regulate this element. While AP-1/NFE-2 elements are necessary for the activity of some erythroid elements, their ability to function independently has not been assessed. In experiments demonstrating their activity they have always been linked to GATA sites (37).

In an attempt to identify cis elements that are sufficient to create active erythroid promoters, we have constructed minimal promoters containing GATA-1, CACCC, and AP-1/NFE-2 motifs and tested their activity in erythroid cells. While none of these elements alone creates an active erythroid promoter, combinations of two elements create active promoters when tested by transient transfection in erythroid cells. GATA-1 motifs linked to either CACCC or AP-1/NFE-2 elements form strong, inducible erythroid promoters. A GATA-1 site bearing a mutation (−175 T → C) from the γ-globin promoter increases expression of these promoters in both fetal and adult erythroid cells. Interaction of these elements thus appears to be sufficient for the formation of active erythroid promoters.

METHODS

Construction of Minimal Promoters. The rabbit β-globin TATA box (positions −10 to −37) was constructed by annealing complementary synthetic oligonucleotides containing *Hin*dIII and *Bam*HI ends (40). This TATA-box fragment was cloned upstream of the human growth hormone (hGH) gene, in the plasmid p0GH (Nichols Institute, San Juan Capistrano, CA), as described (19). Additional double-stranded DNA fragments with minimal promoter elements were similarly constructed by annealing complementary oligonucleotides containing ends with *Hin*dIII overhangs. These included the GATA-1 site from the human ^{A}γ-globin promoter from position −164 to −191 (wild type and −175 T → C HPFH mutation), the CACCC box from both the ^{A}γ-globin promoter (positions −130 to −154) and the human β-globin promoter (positions −80 to −124), and a 46-base-pair (bp) enhancer element containing an AP-1/NFE-2 motif (33, 37). A promoter element containing an altered CACCC sequence from the ^{A}γ-globin promoter (CACCC → TTTAA) was also constructed. Individual and pairs of promoter elements were cloned into the *Hin*dIII site upstream of the rabbit β-globin TATA box. A DNA sequencing primer from the first exon of the hGH gene was used to verify proper orientation of promoter elements.

Cell Culture and Electroporation. The semiadherent adenine phosphoribosyltransferase-negative mouse erythroleukemia (MEL) cell line (41) and a human erythroleukemia (HEL) cell line (HEL-R; ref. 42) were grown in Dulbecco's modified Eagle's medium plus 10% supplemented calf serum (HyClone), in an atmosphere with 5% CO_2. MEL cells were induced to differentiate with 2% (vol/vol) dimethyl sulfoxide following electroporation. Supercoiled plasmid was prepared by purification over a Qiagen tip-500 column (Chatsworth, CA). Electroporations were performed with 20 μg of supercoiled plasmid and 2×10^7 cells in 0.5 ml of 20 mM Hepes, pH 7.05/137 mM NaCl/5 mM KCl/0.7 mM Na_2HPO_4/6 mM dextrose (43). The Bio-Rad Gene Pulser apparatus was used for all electroporations. The electrical field generated was 400 V/cm and 250-μF capacitance for MEL cells, and 300 V/cm and 500-μF capacitance for HEL-R cells. The medium was harvested 60 hr after electroporation, and 100 μl was assayed for hGH by radioimmunoassay (Nichols Institute). Data were analyzed by pooling the hGH levels from three experiments in which all minimal promoter constructs were tested together. Plasmid samples from different bulk preparations were tested in these experiments to overcome any variation due to transfection efficiency as a result of disparity in plasmid preparations. Standard error of the mean was determined for each promoter construct analyzed in both MEL and HEL cells.

RESULTS

We selected GATA, CACCC, and AP-1/NFE-2 elements from the human β-globin locus for use in minimal promoter constructs (Fig. 1). Each construct contained one or more elements upstream of the rabbit β-globin TATA box driving expression of the hGH gene (Fig. 1*D*), and was tested in both a fetal erythroid background (HEL cells) and an adult erythroid background (MEL cells). The HEL cell line used, HEL-R, expresses human ε- and γ-globin chains, while the MEL cell line expresses mouse adult β-globin and will support expression of transfected human β-globin (41, 42). A 46-bp segment from 5′HS2 which contains tandem AP-1/NFE-2 binding sites is shown in Fig. 1*A*. As noted above, this site exhibits erythroid enhancer activity when linked to the γ-globin promoter in transient expression assays and is necessary for the enhancing activity of 5′HS2 (33, 37). The GATA-1 site from the ^{A}γ-globin promoter was also chosen for study (Fig. 1*B*). The wild-type GATA site consists of two GATA motifs which are bound by a single GATA-1 molecule (15). A mutation, T → C, in the proximal GATA motif at position −175 is associated with an HPFH phenotype, in which γ-globin expression is increased in adult-stage red blood cells. This phenotype is dependent on GATA-1 binding (15, 21, 22). GATA-1 and AP-1/NFE-2 motifs were tested singly, and as combinations, by transient transfection in erythroid cells. Neither GATA-1 nor AP-1/NFE-2 binding sites alone create an active erythroid promoter when linked only to a TATA box (Fig. 2 *a–c*). However, when GATA-1 and AP-1/NFE-2 elements are combined, active, inducible erythroid promoters are created (Fig. 2 *f* and *g*). Promoters containing the HPFH GATA site (Fig. 2*g*) are stronger than those containing the wild-type GATA site (Fig. 2*f*) in both MEL and HEL cells. Thus, in minimal promoter constructs consisting only of GATA and AP-1/NFE-2 units, interaction between these elements creates powerful erythroid promoters that express the difference in function caused by a single base change in the GATA site.

To study the interaction between GATA-1 and CACCC binding sites, CACCC elements from both the γ- and β-globin promoters were chosen for study (Fig. 1 *B* and *C*). The element from the γ-globin promoter contains a single CACCC motif at position −141 relative to the mRNA start (cap) site; that from the β-globin promoter contains two CACCC motifs (Fig. 1*C*). Naturally occurring mutations in the β-globin CACCC element are associated with thalassemia syndromes and decreased promoter activity (1–4). Deletion studies of the β-globin promoter have also shown this site to be nec-

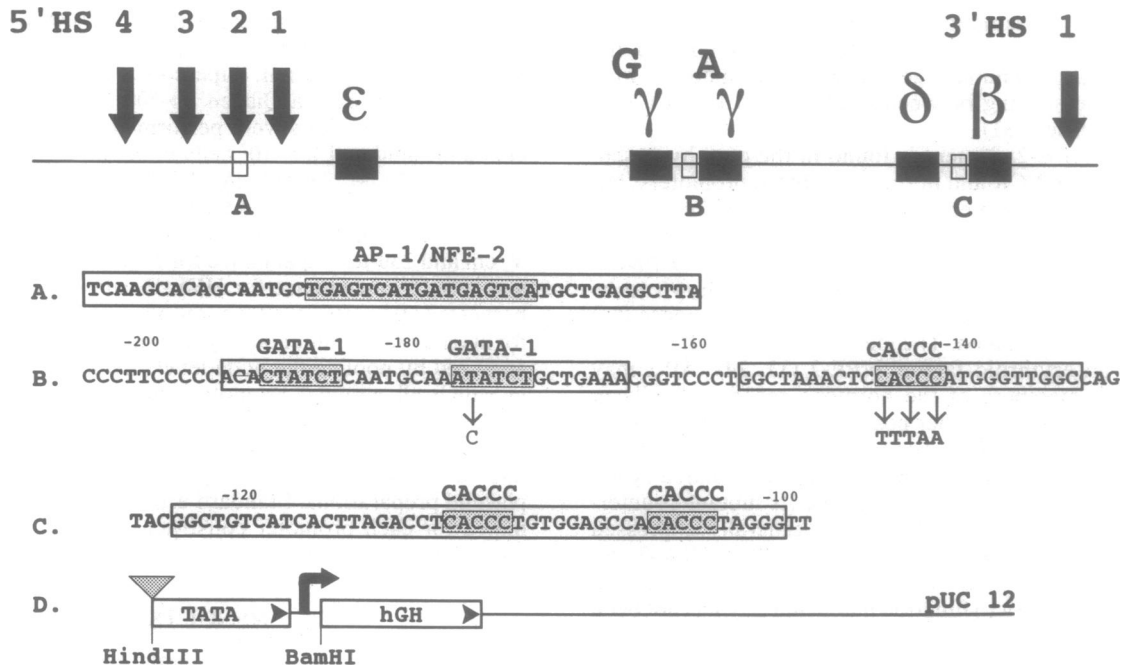

FIG. 1. The human β-globin locus with locations of the cis elements studied (labeled A–C) is shown at the top. (*A*) The 46-bp "core" enhancer from 5'HS2. This contains a tandem AP-1 binding site which is also recognized by the factor NFE-2. This sequence has been shown to contain potent enhancer activity when linked to a γ-globin promoter; "scrambling" the AP-1 sites abolishes enhancer activity. This fragment was utilized in minimal promoter constructs and lacks GATA or CACCC elements. (*B*) The region of the γ-globin promoter containing the CACCC element from position −140 and the GATA-1 site at position −180 relative to the cap site. CACCC and GATA-1 sites are highlighted. The boxed sequences were included in oligonucleotides used in minimal promoter constructs. The −175 T→C mutation associated with HPFH is indicated. A mutation introduced into the CACCC sequence is denoted by arrows below the CACCC box. The native distance between GATA and CACCC elements shown here was duplicated in minimal promoter constructs. (*C*) The CACCC elements from the β-globin promoter are highlighted. The boxed sequence was used in minimal promoter constructs and lacks a GATA-1 binding site. (*D*) The plasmid used to assay transcriptional activation by the oligonucleotides shown in *A–C*. These are cloned (in the arrangements shown in Fig. 2) upstream of a rabbit β-globin TATA box driving expression of the hGH gene.

essary for promoter activity (1). The element we used in these experiments has been reported to include a GATA-1 binding site at position −118. However, we were unable to detect any GATA-1 binding to this site in a gel retardation assay (data not shown). The GATA-1 sites from the γ-globin promoter (wild type and HPFH) were combined with each CACCC element and tested in both MEL and HEL cells. Neither CACCC element alone creates an active erythroid promoter (Fig. 2 *d* and *e*). When the wild-type GATA site is linked to either CACCC element, the promoters are also inactive (Fig. 2 *i* and *j*). However, linkage of the HPFH GATA site to the CACCC element from either the γ- or the β-globin promoter creates an inducible promoter (Fig. 2 *k* and *l*) that is active in both MEL and HEL cells. When the CACCC motif from the γ-globin promoter is mutated to TTTAA, the activity is lost (Fig. 2*m*). The inactivity of the wild-type GATA/CACCC constructs is reminiscent of transient transfection experiments previously performed with the $^{A}\gamma$ promoter (15). In those experiments, deletion of the wild-type GATA-1 site had no effect on promoter strength in erythroid cells. However, when the HPFH GATA site was deleted, the 5-fold-increased promoter strength associated with the HPFH mutation was lost. The result obtained when either GATA site is linked to β-globin promoter CACCC element (Fig. 2 *j* and *l*) is the same as with the γ-globin CACCC element, with one notable exception. In cells expressing fetal globin (HEL cells) the HPFH GATA/γ-CACCC construct is more active than the HPFH GATA/β-CACCC construct, whereas in cells expressing adult globin (MEL cells), the HPFH GATA/β-CACCC construct is more active than the HPFH GATA/γ-CACCC construct.

The AP-1/NFE-2 element was also combined with globin promoter CACCC elements. As already noted, neither element alone creates an active erythroid promoter (Fig. 2 *a*, *d*, and *e*). However, when the AP-1/NFE-2 motif is combined with the CACCC element from the β-globin promoter (Fig. 2*h*) or the γ-globin promoter (data not shown) activity is high in both MEL and HEL cells. Thus, combinations of GATA, CACCC, and AP-1/NFE-2 motifs create promoters which are active in transient assays in MEL and HEL cells.

DISCUSSION

We have shown that three cis-acting elements from the human β-globin locus form strong promoters when linked to each other, but not alone. These promoters are not of uniform activity; variation in the sequence of either GATA or CACCC elements creates differences in activity that appear to correlate with their behavior *in vivo*. Our results suggest that the minimal promoters have characteristics like those of the native promoters from which their components are derived, so that this method may be useful for analyzing the function and interaction of the individual components of promoters and enhancers.

Our evidence strongly suggests that interaction of GATA-1 with other factors is crucial for regulation of globin expression by GATA-1. This suggests a mechanism for the action of GATA-1 on genes expressed in distinct lineages. If GATA-1 interacts with a wide variety of factors, promoters containing a functional GATA-1 site may be active only when factors that interact with GATA-1 are present. In other hematopoietic lineages expressing GATA-1 (mast cells and megakaryocytes), factors recognizing erythroid CACCC and AP-1/NFE-2 elements may not be expressed, thus passively down-regulating erythroid genes. Conversely, in megakaryocyte/mast cell promoters, GATA-1 sites may be flanked by

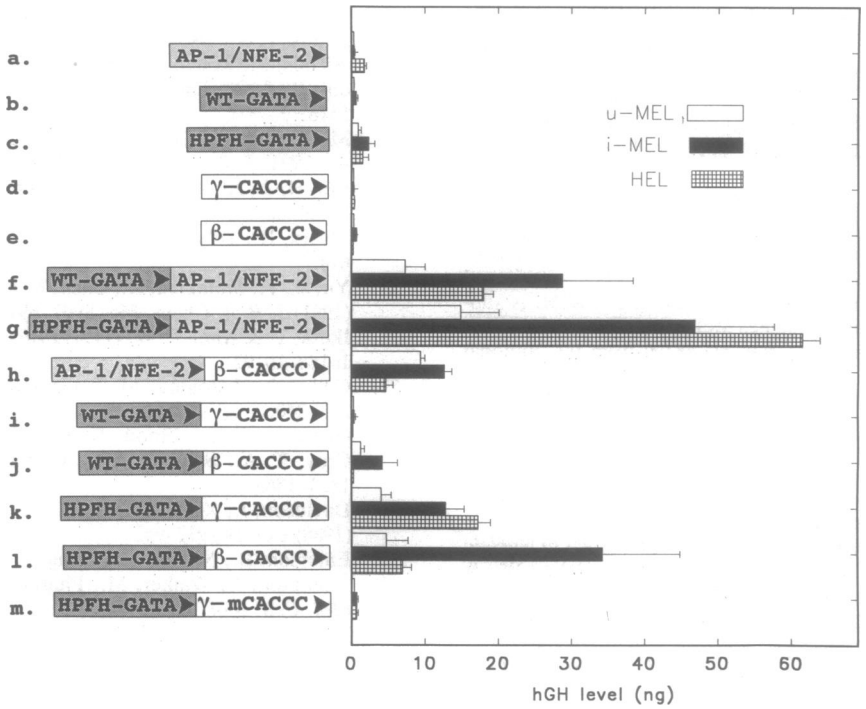

FIG. 2. Transient transfections in erythroid cells. MEL cells, which express mouse β-major globin when induced with dimethyl sulfoxide, and HEL cells, which constitutively express human ε and γ-globin, were transfected by electroporation with the indicated minimal promoter constructs. hGH levels in culture medium are indicated by bars. Open bars denote electroporations done with uninduced MEL cells, solid bars, MEL cells induced with 2% dimethyl sulfoxide, and crosshatched bars, HEL cells. These results consist of averages of three separate experiments in which all constructs were tested in duplicate. Error bars represent standard error of the mean. Constructs are shown schematically on the left; the AP-1/NFE-2 box corresponds to the element described in Fig. 1*A*; WT-GATA, HPFH-GATA, γ-CACCC and γ-mCACCC boxes are indicated in Fig. 1*B*; the β-CACCC box is shown in Fig. 1*C*.

elements bound by a different set of factors, which interact with GATA-1 to activate these genes in mast cells and megakaryocytes. This would effect tissue-specific regulation by GATA-1 of two different sets of genes. More knowledge of the functional elements of megakaryocyte/mast cell promoters regulated by GATA-1 will be required to test this hypothesis.

Our results also suggest a reevaluation of the cotransfection experiments which demonstrated transcriptional activation by GATA-1 and mapped an acidic activation domain at its amino terminus (18, 19). As mentioned above, minimal GATA promoters, while active, were not responsive to changes in the number and type of GATA site. While activation of those promoters in nonerythroid cells is dependent on the acidic domain, interaction with other factors may require domains that are as yet unmapped. A similar conclusion may be drawn with respect to the factors GATA-2 and GATA-3, both of which have been shown to activate the same minimal GATA promoters (44).

The ability of GATA-1 to distinguish between wild-type and HPFH sites most likely involves the DNA-binding domain but was not seen with GATA promoters in nonerythroid cells. That the HPFH phenotype is expressed when the site is linked to either a CACCC or a AP-1/NFE-2 element argues that the effect is intrinsic to the GATA-1/DNA interaction and is merely amplified by linkage to the other elements. The basis for the overactivity of the −175 T → C mutant remains unknown. No difference in affinity of the two sites for GATA-1 was found in previous studies or in a recent comparison of their equilibrium dissociation constants (D.I.K.M., unpublished observation).

Our data imply that globin gene regulation is achieved by the combined action of several transcription factors, as has been reported with other systems (46). It is not possible to conclude from this work which of the elements we examined is acted on by erythroid-specific factors, as each is silent alone and active in concert with the others in red cells. GATA elements, however, are clearly bound by GATA-1, which is known to be expressed only in erythroid, megakaryocytic, and mast cells. Since both AP-1/NFE-2 and CACCC elements are active in nonerythroid cells (unpublished data), it is tempting to speculate that the erythroid activity of these promoters may be provided by GATA-1. However, both AP-1/NFE-2 and CACCC motifs have been reported to bind erythroid nuclear factors (30, 33, 34, 36). Generally expressed factors also bind both elements, complicating any interpretation of their activity in an erythroid environment.

The AP-1/NFE-2 element is a strong positive regulator of erythroid gene expression (35–37). We have shown that its action is weak unless linked to another element. An erythroid factor, NFE-2, has been described as binding this element (33, 34). It has not been demonstrated, however, that this factor is responsible for the full activity of the AP-1/NFE-2 element. On the basis of the known interaction between AP-1 and members of the hormone receptor family (38, 39, 45), as well as the described regulation of an NFE-2 site by c-ErbA (the thyroid hormone receptor) (35), we speculate that AP-1/NFE-2 elements are composite hormone response elements (46). They may thus respond to AP-1 as well as to one or more members of the hormone receptor family; these might be c-ErbA and a retinoic acid receptor. If this speculation is correct, NFE-2 may be only one of a set of factors acting on the AP-1/NFE-2 element, and the contribution of each factor to the function of the element will be very difficult to assess.

The CACCC elements present a similar picture. Numerous factors have been shown to bind them, but which factor acts on erythroid CACCC elements *in vivo* is unclear, and it is possible that more than one does so. Our data suggest functional dissimilarities between distinct CACCC elements.

Our results strongly suggest that not all GATA sites are functionally equivalent; AP-1/NFE-2 and CACCC sites may exhibit functional heterogeneity as well. A question raised by these observations is whether this variability influences erythroid gene regulation. Investigation of this issue may uncover some of the subtleties of erythroid gene transcription.

We thank Mark Groudine, Chul Kim, Elliot Epner, and George Stamatoyannopoulos for helpful discussions and critical review of the manuscript. M.W. is supported in part by Grants CA09351 and CA09645 from the National Institutes of Health. D.I.K.M. is supported by a scholarship from the James McDonnell Foundation. This work was supported in part by Grant HL48790 from the National Institutes of Health.

1. Evans, T., Felsenfeld, G. & Reitman, M. (1990) *Annu. Rev. Cell. Biol.* **6**, 95–124.
2. Stamatoyannopoulos, G., Nienhuis, A., Leder, P. & Majerus, P., eds. (1986) *The Molecular Basis of Blood Diseases* (Saunders, Philadelphia), pp. 28–105.
3. Collins, F. S. & Weissman, S. M. (1984) in *Progress in Nucleic Acid Research and Molecular Biology*, eds. Cohn, W. & Moldave, K. (Academic, Orlando, FL), Vol. 31, pp. 315–462.
4. Karlsson, S. & Nienhuis, A. W. (1985) *Annu. Rev. Biochem.* **54**, 1071–1108.
5. Chada, K., Magram, J. & Constantini, F. (1986) *Nature (London)* **319**, 685–687.
6. Collis, P., Antoniou, M. & Grosveld, F. (1990) *EMBO J.* **9**, 233–240.
7. Forrester, W. C., Thompson, C., Elder, J. T. & Groudine, M. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 1359–1363.
8. Tuan, D., Solomon, W., Li, Q. & London, I. M. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6384–6388.
9. Forrester, W. C., Epner, E., Driscoll, M. C., Enver, T., Brice, M., Papayannopoulou, T. & Groudine, M. (1990) *Genes Dev.* **4**, 1637–1649.
10. Choi, O. B. & Engel, J. D. (1988) *Cell* **55**, 17–26.
11. Enver, T., Raich, N., Ebens, A., Papayannopoulou, T. & Stamatoyannopoulos, G. (1990) *Nature (London)* **344**, 309–313.
12. Behringer, R., Ryan, T., Palmiter, R., Brinster, R. & Townes, T. M. (1986) *Genes Dev.* **4**, 380–389.
13. Barnhart, K., Kim, C. & Shefferey, M. (1989) *Mol. Cell. Biol.* **9**, 2606–2614.
14. Catala, F., deBoer, E., Habets, G. & Grosveld, F. (1989) *Nucleic Acids Res.* **17**, 3811–3827.
15. Martin, D. I. K., Tsai, S.-F. & Orkin, S. H. (1989) *Nature (London)* **338**, 435–438.
16. Martin, D., Zon, L., Mutter, G. & Orkin, S. (1990) *Nature (London)* **344**, 444–446.
17. Romeo, P.-H., Prandini, M.-H., Joulin, V., Mignotte, V., Prenant, M., Vainchenker, W., Marguerie, G. & Uzan, G. (1990) *Nature (London)* **344**, 447–449.
18. Pevny, L., Simon, M., Robertson, E., Klein, W., Tsai, S.-F., D'Agati, V., Orkin, S. & Constantini, F. (1991) *Nature (London)* **349**, 257–260.
19. Martin, D. & Orkin, S. (1990) *Genes Dev.* **4**, 1886–1898.
20. Evans, T. & Felsenfeld, G. (1991) *Mol. Cell. Biol.* **11**, 843–853.
21. Nicolis, S., Ronchi, A., Malgaretti, N., Mantovani, R., Giglioni, B. & Ottolenghi, S. (1989) *Nucleic Acids Res.* **17**, 5509–5516.
22. McDonagh, K., Lin, H., Lowrey, C., Bodine, D. & Nienhuis, A. (1991) *J. Biol. Chem.* **266**, 11965–11974.
23. Lin, H. J., Anagnou, N. P., Rutherford, T. R., Shimada, T. & Nienhuis, A. W. (1987) *J. Clin. Invest.* **80**, 374–380.
24. Myers, R., Tilly, K. & Maniatis, T. (1986) *Science* **232**, 613–618.
25. Yu, C.-Y., Motamed, K., Chen, J., Bailey, A. & Shen, J. C.-K. (1991) *J. Biol. Chem.* **266**, 8907–8915.
26. Ikuta, T. & Kan, Y. W. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 10188–10192.
27. Collis, P., Antoniou, M. & Grosveld, F. (1990) *EMBO J.* **9**, 233–240.
28. Gumucio, D. L., Rood, K. L., Blanchard-McQuate, K. L., Gray, T. A., Saulino, A. & Collins, F. S. (1991) *Blood* **78**, 1853–1863.
29. Davidson, I., Xiao, J., Rosales, R., Staub, A. & Chambon, P. (1988) *Cell* **54**, 932–942.
30. Emerson, B., Nickol, J. & Fong, T. (1989) *Cell* **57**, 1189–1200.
31. Frampton, J., Walker, M., Plumb, M. & Harrison, P. (1990) *Mol. Cell. Biol.* **10**, 3838–3842.
32. Mignotte, V., Wall, S., deBoer, E., Grosveld, F. & Romeo, P. (1989) *Nucleic Acids Res.* **17**, 37–54.
33. Ney, P., Sorrentino, B., Lowrey, C. & Nienhuis, A. (1990) *Nucleic Acids Res.* **18**, 6011–6017.
34. Philipsen, S., Talbot, D., Fraser, P. & Grosveld, F. (1990) *EMBO J.* **9**, 2159–2167.
35. Disela, C., Glineur, C., Bugge, T., Sap, J., Stengl, G., Dodgson, J., Stunnenberg, H., Beug, H. & Zenke, M. (1991) *Genes Dev.* **5**, 2033–2047.
36. Mignotte, V., Wall, L., deBoer, E., Grosveld, F. & Romeo, P. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 6548–6552.
37. Ney, P., Sorrentino, P., McDonagh, K. & Nienhuis, A. (1990) *Genes Dev.* **4**, 993–1006.
38. Schüle, R., Umesono, K., Mangelsdorf, D., Bolado, J., Pike, J. & Evans, R. M. (1990) *Cell* **61**, 497–504.
39. Sap, J., Vennström, B. & Beug, H. (1990) *Cell* **61**, 1035–1049.
40. Wester, G., Gerster, T., Müller, M., Schaffner, G. & Schaffner, W. (1987) *Nucleic Acids Res.* **15**, 6787–6798.
41. Charnay, P., Treisman, R., Mellon, P., Chao, M., Axel, R. & Maniatis, T. (1984) *Cell* **38**, 251–263.
42. Papayannopoulou, T., Nakamoto, B., Kurachi, S. & Nelson, R. (1987) *Blood* **70**, 1764–1772.
43. Chu, G., Hayakawa, H. & Berg, P. (1987) *Nucleic Acids Res.* **15**, 1311–1326.
44. Yamamoto, M., Ko, L., Leonard, M., Beug, H., Orkin, S. & Engel, J. (1990) *Genes Dev.* **4**, 1650–1662.
45. Privalsky, M., Sharif, M. & Yamamoto, K. (1990) *Cell* **63**, 1277–1286.
46. Schüle, R. & Evans, R. M. (1991) *Trends Genet.* **7**, 377–381.
47. Strauss, E. & Orkin, S. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 5809–5813.