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Regulation of the myeloperoxidase enhancer binding proteins Pu1, C-EBPα, -β, and -δ during granulocyte-lineage specification

(chromatin/enhancers/hemopoietic stem cells/transcription factor phosphorylation)

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ABSTRACT **We have compared the molecular architecture and function of the myeloperoxidase upstream enhancer in multipotential versus granulocyte-committed hematopoietic progenitor cells. We show that the enhancer is accessible in multipotential cell chromatin but functionally incompetent before granulocyte commitment. Multipotential cells contain both Pu1 and C-EBPα as enhancer-binding activities. Pu1 is unphosphorylated in both multipotential and granulocyte-committed cells but is phosphorylated in B lymphocytes, raising the possibility that differential phosphorylation may play a role in specifying its lymphoid versus myeloid functions. C-EBPα exists as multiple phosphorylated forms in the nucleus of both multipotential and granulocyte-committed cells. C-EBPβ is unphosphorylated and cytoplasmically localized in multipotential cells but exists as a phosphorylated nuclear enhancer-binding activity in granulocyte-committed cells. Granulocyte colony-stimulating factor-induced granulocytic differentiation of multipotential progenitor cells results in activation of C-EBPδ expression and functional recruitment of C-EBPδ and C-EBPβ to the nucleus. Our results implicate Pu1 and the C-EBP family as critical regulators of myeloperoxidase gene expression and are consistent with a model in which a temporal exchange of C-EBP isoforms at the myeloperoxidase enhancer mediates the transition from a primed state in multipotential cells to a transcriptionally active configuration in promyelocytes.**

While the molecular mechanisms of lineage choice remain largely unknown, the functional balance of transcription factors and the accessibility of cis-acting DNA regulatory elements are likely to be important components in lineage determination (1, 2). The β-globin and immunoglobulin loci have long served as models for erythroid and lymphoid specific transcriptional regulation, respectively, but considerably less is known about the molecular control of myeloid-specific locus activation.

To gain further insight into the early steps involved in commitment and gene activation in myeloid cell differentiation, we have been analyzing the transcriptional regulation of the myeloperoxidase (MPO) gene, whose expression is restricted to the promyelocyte stages of granulocytic differentiation (3, 4). We have recently identified a myeloid lineage-specific enhancer ≈3.1 kb upstream of the murine MPO gene that resides in a DNase I hypersensitive region (5′HS4) in myeloid cells but lies within inaccessible chromatin in nonmyeloid cell types (5). An important question is how the accessibility and functional status of this upstream enhancer are developmentally linked to both granulocyte lineage commitment and subsequent stable expression of MPO mRNA and protein specifically in promyelocytes.

We have now addressed this issue by using multipotential hemopoietic stem cells that retain granulocyte differentiation potential (6, 7) to analyze the chromatin structure and transcription factor status of the MPO gene before commitment to the granulocyte lineage. To our knowledge, our results provide the first detailed comparison of the architecture of a lineage-specific enhancer in stem versus myeloid-committed cells.

MATERIALS AND METHODS

Cells and Cell Lines. The multipotential interleukin 3-dependent FDCP-mix A4 cells (A4 or multipotential progenitors) (7), a kind gift of E. Spooncer (Manchester, U.K.); the WEHI-3B D+ cell line (8), referred to as D+ or granulocyte precursor; the pre-B cell line 18.8 (9), a kind gift of F. Alt (Boston), and the EL4 cell line (pre-T) (10) ATCCTIB39 were maintained as described (11). The interleukin 3-dependent cells 32D cl3, referred to as 32D or progenitor cells (12), a kind gift of J. Greenberger (Pittsburgh), were maintained in RPM1 1640 medium (GIBCO) that was supplemented with 10% fetal calf serum and 10% WEHI-conditioned medium. A4 cells were induced to granulocytes essentially as described (4), except that recombinant granulocyte colony-stimulating factor (G-CSF) was used in place of lung-conditioned medium.

Chromatin Structure and Functional Analysis. For DNase I hypersensitive site analyses, purified nuclei were treated with increasing amounts of DNase I as previously described (5). Transient transfections were also performed essentially as described (5). The strength of enhancer activity was measured as fold enhancement over the luciferase activity of the control plasmid (Pt-109) alone and the mean values of enhancer activity were derived from five separate transfection assays each carried out with five different plasmid preparations.

Preparation of Nuclear Extracts and Electrophoretic Mobility-Shift Assays (EMSAs). Nuclear extracts were prepared from exponentially growing cells by the method of Schreiber *et al.* (13), as modified by Wilson *et al.* (14) to include a range of protease inhibitors. Extracts were aliquoted and stored at −70°C or used immediately.

EMSAs were carried out on ice for 20 min in reaction mixtures of 20 μl that contained 10 μg of nuclear extract, 2.5 fmol ^{32}P-radiolabeled double-stranded oligonucleotide, 1 μg of poly(dI·dC), 10 mM Tris·HCl (pH 7.5), 50 mM NaCl, 0.5 mM DTT, 10% glycerol, 0.05% Nonidet P-40, 2 mM EDTA, and 300 μg/ml BSA. In competition experiments, a 100-fold molar excess of unlabeled wild-type or mutated oligonucleotide was added to the reaction mix for 20 min before the addition of radiolabeled probe. Protein–DNA complexes were separated on 5% nondenaturing polyacrylamide gels in 0.25× TBE buffer at room temperature. The upper strand sequence of each annealed oli-

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Abbreviations: MPO, myeloperoxidase; G-CSF, granulocyte colony-stimulating factor; EMSA, electrophoretic mobility-shift assay.

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gomer used is shown below. Specific polyclonal anti-sera to Pu1, Ets1/2, C-EBPα, C-EBPβ, and C-EBPδ were obtained from Santa Cruz Biotechnology. For antibody supershift assays, 1 μl of a particular antibody was added to the reaction mix and incubated for 40 min on ice before the addition of radiolabeled probe.

Western Blot Analysis. Nuclear protein extracts were prepared as described for EMSAs. For whole cell protein extracts, 3×10^6 exponentially growing cells were prepared essentially as described (15). The supernatants were frozen at −70°C or used immediately and boiled in Laemelli buffer before being loaded onto a 15% acrylamide, 0.16% bisacrylamide SDS/PAGE gel. After electrophoresis, proteins were transferred to polyvinylidene difluoride membranes (Millipore) by semi-dry transfer. Membranes were blocked for 60 min in TBS with 0.1% Tween 20 (TBS-T) containing 5% (wt/vol) milk powder as blocking agent. Specific antibodies (polyclonal rabbit; Santa Cruz Biotechnology) were incubated with the membranes for 45 min at room temperature in the presence of fresh blocking buffer. After two 10-min washes in TBS-T, membranes were incubated for 60 min in the presence of a 1:2500 dilution of peroxidase-linked donkey anti-rabbit antibody (Amersham) in blocking buffer. After one wash of 15 min followed by four washes of 5 min each in TBS-T, membranes were developed by the Enhanced Chemiluminescence detection kit (Amersham).

Immunofluorescent Staining of Cells. Cytospins were dried, fixed in ice-cold acetone for 2 min, dried again, and rehydrated in PBS. Slides were blocked in 10% fetal calf serum for 30 min at room temperature, washed with PBS, and incubated overnight at 4°C with 10 μg/ml isoform-specific C-EBP polyclonal antibodies. Cytospins were washed in PBS and incubated with fluorescein isothiocyanate-conjugated $F(ab')_2$ fragments of goat anti-rabbit immunoglobulins for 45 min at room temperature before mounting and examination on an MRC 600 confocal microscope (Bio-Rad).

RESULTS

The Upstream MPO Enhancer Is Primed for Activation but Is Inactive in Progenitor Cells. When under interleukin 3-driven proliferating conditions, multipotential FDCP-mix A4 (A4) cells (4) and 32D cl3 (12) granulocyte progenitor cells normally express little if any MPO RNA as detected by Northern blot analysis. However, nuclease hypersensitive site analysis revealed that the myeloid-specific enhancer hypersensitive site (5′HS4, Fig. 1*A*) was already established in A4 and 32D cells, suggesting that the enhancer may be partially activated before commitment to the granulocyte lineage (Fig. 1*B*). The enhancer region remains open in granulocyte-induced progeny at day 5 of differentiation when the MPO gene is being maximally expressed (Fig. 1*B*). We therefore assessed the functional status of the enhancer in A4 cells by transient transfection. A reporter gene, pSc/K-luc (5), containing the minimal upstream enhancer linked to the herpes simplex virus thymidine kinase-luciferase gene (Pt-109) was transfected into A4 progenitor cells, control granulocytic cells [WEHI-3BD+ (D+)], and control lymphoid cells (18.8). In these experiments, 5′HS4 exhibited greater than 10-fold enhancer effect in granulocytic cells but showed little or no enhancer activity in A4 cells or control lymphoid cells (Fig. 1*C*). These results suggest that although it is accessible to nuclear transcription factors in multipotential progenitor cells, the MPO enhancer is not functionally active before granulocytic commitment.

Pu1 Binds the MPO Enhancer in Multipotential and Lineage-Committed Cells. The MPO enhancer consists of at least three cis-acting elements (FTI, FTII, and FTIII; refs. 5 and 16). Nuclear proteins binding to FTI were detected in extracts from myeloid and pre-B cells but not from pre-T cells (Fig. 2), suggesting that this interaction, in which the protected region contains two repeats of the sequence GGAA and resembles the purine-rich sequence bound by members of the Ets family, may involve the binding of the Ets-related transcription factor Pu1 (17). Oligonucleotides with mutations within the protected region were analyzed for their ability to compete for the complex. Substitution of two G residues in the second (2) but not the first (1) potential binding site rendered the motif unable to compete for binding of the factor (Fig. 2*A*), thus confirming that the protein binds to the second GGAA motif. These sequences exactly match a recently derived consensus recognition site for Spi1/Pu1 (18). Involvement of Pu1 in the complex was confirmed by antibody supershift analysis; the complex is retarded by anti-Pu1 antiserum but not by pre-immune or anti-ets1/ets2 antisera (Fig. 2*B*). Nuclear proteins from A4 progenitor cells also bound this motif and showed a supershift only with anti-Pu1 serum (Fig. 3*A*) as did extracts from 32D cells (data not shown). This result demonstrates the presence

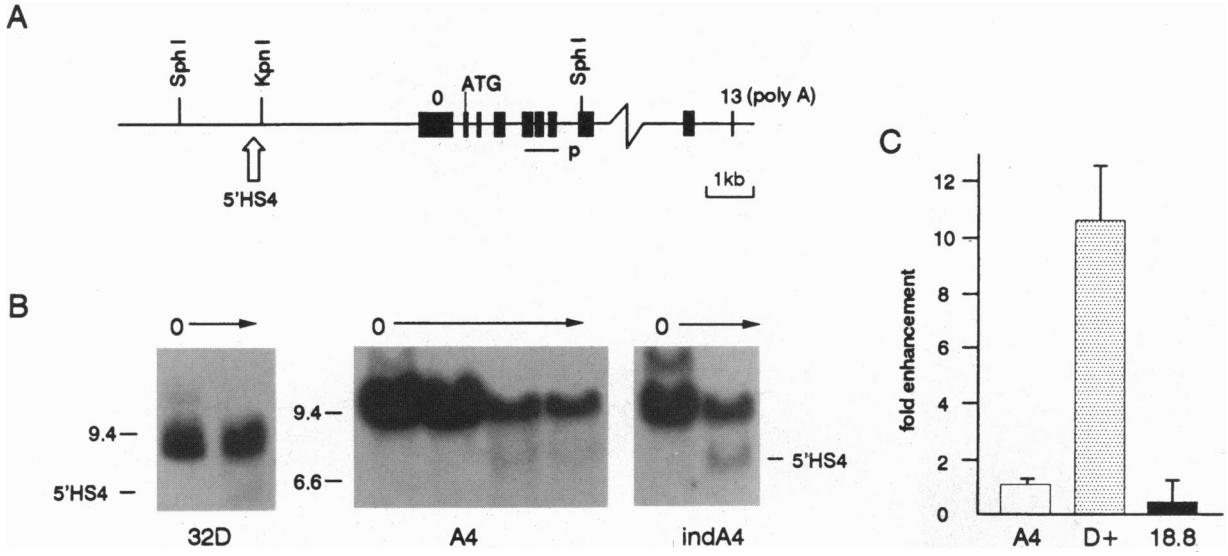

FIG. 1. The MPO enhancer is accessible but nonfunctional before granulocytic commitment. (*A*) Diagram showing the location of 5′HS4, the enhancer of the murine MPO locus. Data modified from ref. 5. p, MPO cDNA probe corresponding to exons 4–6. (*B*) DNA (15 μg) from nuclei digested with no (0) or increasing amounts of DNase I was restricted with *Sph*I and subjected to Southern blotting with probe p. 32D and A4 represent granulocyte- and multipotential-progenitor cells, respectively. indA4, A4 cells 5 days after induction with G-CSF. (*C*) Transient transfection assays were performed using the minimal enhancer linked to a thymidine kinase-luciferase reporter gene. Plotted values represent the mean derived from five separate transfection assays each carried out with five different plasmid preps. D+, MPO-expressing granulocyte-precursor (myelomonocyte) cells; 18.8, pre-B lymphoid cells.

of Pu1 protein in multipotential progenitor cells as a DNA-binding activity before commitment.

Pu1 is known to bind DNA in a phosphorylation-independent manner but phosphorylation is required for its interaction with the B cell-restricted factor NF-EM5 and for its transcriptional activity (19). We compared Pu1 phosphorylation status in A4 cells and granulocyte precursor cells by Western blot analysis of low bis-acrylamide gels in which phosphorylated Pu1 appears as a band of retarded mobility (15) The phosphorylation status of these differently migrating forms was confirmed by electrophoretic analysis of immunoprecipitated Pu1 protein labeled *in vivo* by [^{32}P]orthophosphate (data not shown). Strikingly, Pu1 is nonphosphorylated in both A4 and D+ cells compared with its phosphorylated form in 18.8 pre-B cells (Fig. 3*B*).

The MPO Enhancer Contains Multiple Binding Elements for the C-EBP Family of Transcription Factors. The FTII region contains three adjacent potential binding sites for the C-EBP family of leucine-zipper proteins that bind two similar cis-regulatory elements: T($^{T}/_{G}$)NNGNAA($^{G}/_{T}$) (20) and ($^{G}/_{C}$)AAT, which is a directly abutting half-site with dyad-symmetry (21). Fig. 4 shows nuclear protein binding to FTII in myeloid cells and a supershift of the complex with anti-sera to C-EBPβ. A mutation in the core of the first or second site (MT1 and MT2) is still able to compete for binding of the complex. Similarly, a mutation in the core region of two sites (MT3 and MT4) can still compete for the complex, presumably because the third motif is intact. An oligonucleotide that contains all three mutations (MT5) is now unable to compete for binding. The protected region of FTIII contains an almost identical motif (TGAGGCAAC) to that seen to bind C-EBP in FTII. FTIII also binds C-EBP isoforms in myeloid cells and the complex is supershifted by anti-sera to C-EBPβ (Fig. 4). Furthermore, an oligonucleotide from FTIII can compete for binding at FTII and one from FTIII for binding at FTII, again showing that the complexes are similar.

Differential Binding of C-EBP Family Isoforms to the MPO Enhancer. To characterize C-EBP binding at the MPO enhancer more precisely, we first analyzed the profile of isoform binding in MPO-expressing D+ cells. Preincubation of the complex formed on FTII in D+ cells with antisera to C-EBPα, -β, and -δ gave supershifts of the β- and δ-complexes and, to a lesser extent, that of C-EBPα (Fig. 5). These data confirmed the binding of different isoforms of the C-EBP family to FTII. The faint complex seen in pre-B cells (18.8) does not supershift with any of the anti-sera used but does compete with cold self DNA and probably reflects binding of C-EBPγ, which is abundant in B cells (22). We next compared the pattern of isoform expression in D+ granulocyte precursor cells to that

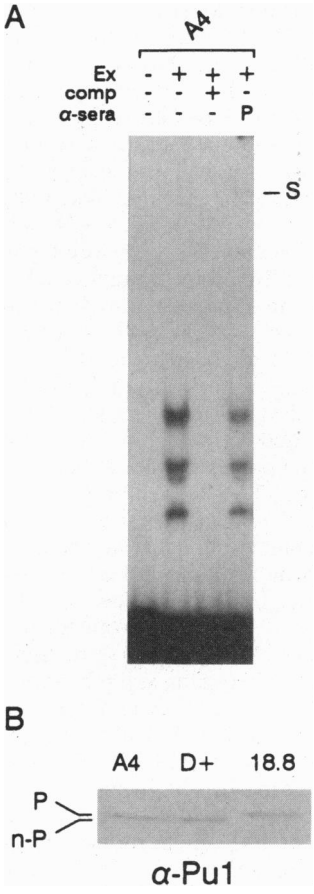

FIG. 3. Distribution and phosphorylation status of Pu1. (*A*) Nuclear protein extracts isolated from A4 cells were subjected to EMSA as described above. P, incubation of extracts with anti-sera to Pu1 before addition of FTI probe; S, the Pu1 supershift. (*B*) Cells (3×10^6) were subjected to Western blot analysis in low bis-acrylamide SDS/PAGE gels and transferred to polyvinylidene difluoride membranes. After blocking, blots were incubated in anti-sera to Pu1 and assayed by chemiluminescence. P, the slow migrating, phosphorylated form of Pu1; h-P, the faster migrating hypophosphorylated form of the protein (see text).

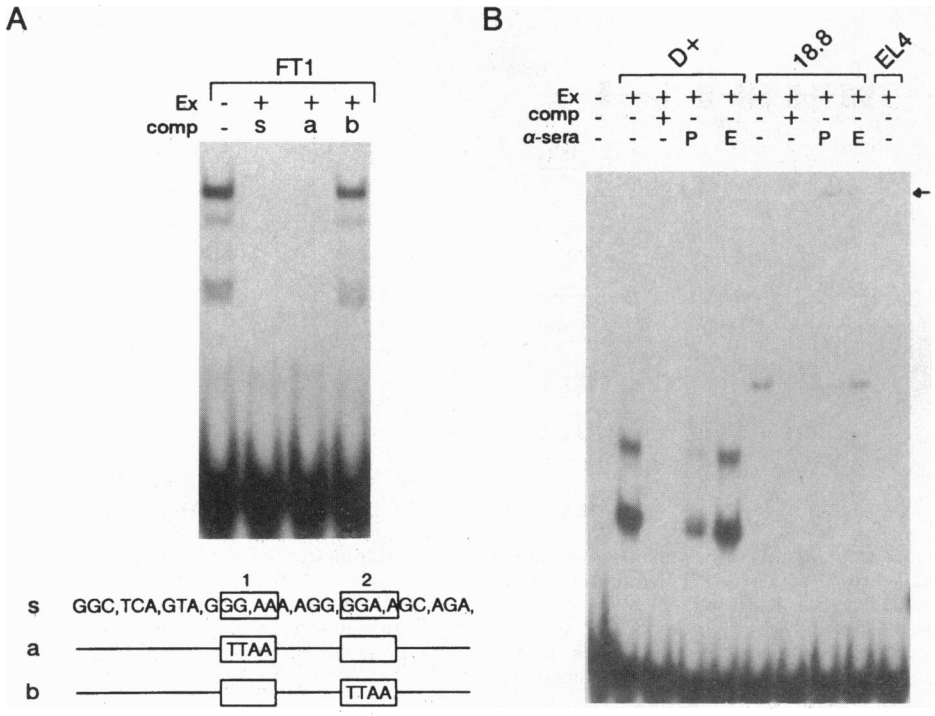

FIG. 2. Characterization of proteins binding to FTI. (*A*) DNA binding assays were performed with ^{32}P-labeled FTI oligonucleotide in the presence of myeloid cell nuclear extracts (Ex). Competition assays (Comp) were done with unlabeled self (s) or mutated (a and b) oligonucleotides. All competitors were used in 100-fold molar excess. (*B*) Nuclear extracts from D+ (granulocyte), 18.8 (pre-B), and EL4 (pre-T) cells were incubated in the presence of anti-sera to Pu1 (P) or Ets1/Ets2 (E) for 1 hr before addition of labeled FTI. Even in the presence of the major protease inhibitors, some proteins in myeloid cell extracts are extremely prone to proteolysis giving rise to a faster migrating complex. An arrow marks a supershift of Pu1.

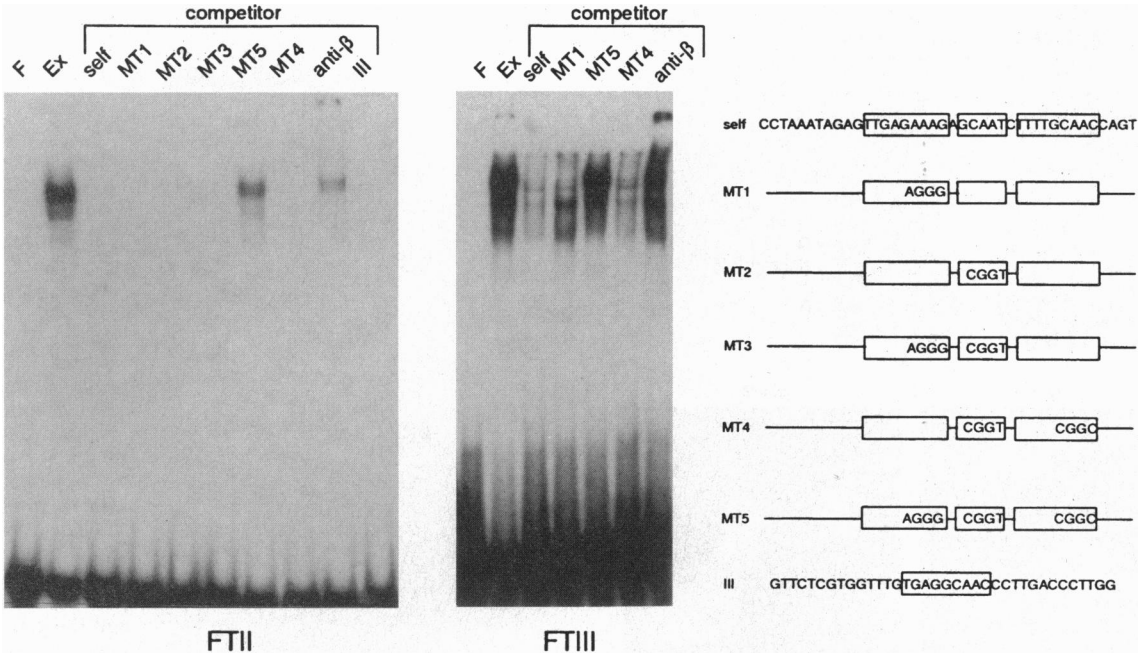

FIG. 4. Characterization of proteins binding to FTII and FTIII. (*Left*) Competitive DNA binding assays were carried out as described above. Radiolabeled FTII was cold-competed using the wild-type and mutated oligonucleotides shown at *Right*. F, free probe. (*Center*) Competitive binding assays were performed on FTIII as described for FTII above. (*Right*) The elements containing potential binding sites for C-EBP are shown in boxes. In the mutated oligonucleotides, only the individually mutated bases are shown and compared with wild-type (self). III, the wild-type sequence of FTIII.

found in earlier progenitor cells. In contrast to the supershifts detected in granulocyte precursor cells, a more significant supershift was seen with extracts from 32D cells using anti-sera to C-EBPα and to a lesser extent C-EBPβ. However, no supershift was seen of C-EBPδ complexes (Fig. 5), suggesting that C-EBPδ is either absent or resides in the nucleus in an inactive, non-DNA binding form. In contrast, in the earlier multipotential A4 cells, only the C-EBPα complex was detected (Fig. 6 *Upper*). EMSAs performed 2 days after granulocyte induction of A4 cells with G-CSF show the relative loss of the supershift with anti-sera to C-EBPα, coupled with the appearance of both C-EBPβ and C-EBPδ isoforms (Fig. 6), i.e., a pattern of isoform binding almost identical to that seen in the granulocyte-committed D+ cells.

Different Cellular Localization and Phosphorylation Status of C-EBP Isoforms in Multipotential and Myeloid-Committed Cells. We next assayed the cellular localization and functional (phosphorylation) status of these factors in multipotential versus myeloid-induced hematopoietic cells by immunofluorescence and Western blot analyses. C-EBPβ activity was not revealed by EMSA using day 0 nuclear extracts of A4 (Fig. 6 *Upper*) and could not be detected in the nucleus by immunofluorescence (Fig. 6 *Lower*). However, strong staining of C-EBPβ was seen in the cytoplasm of these cells. This pattern of C-EBPβ staining changed dramatically after G-CSF induction. The expression of C-EBPβ in the cytoplasm is markedly reduced from that seen before G-CSF addition. In contrast, the nucleus that was devoid of C-EBPβ staining in undifferentiated cells now contains significant levels. While the nuclear/cytoplasmic ratio of C-EBPβ has changed dramatically, it should be noted that the overall level of C-EBPβ in day 2 cells appears to be somewhat lower than that present in the day 0 population.

Western blotting shows that C-EBPα, which exists as two major alternative translation products, p42α and p30α (23), is present in the nucleus of both A4 and D+ cells (Fig. 7*A*). Hence, there is no detectable difference in the C-EBPα isoform detected in progenitor or granulocyte-committed cell types. We next examined the phosphorylation state of C-EBPβ, which also exists as two alternative translation products of p38β and p23β (LAP and LIP, respectively) (21). Again consistent with the EMSA data, C-EBPβ was found to be present in the nucleus of only D+ cells and not in A4 cells (Fig. 7*B*). Strikingly, an analysis of total cell extracts prepared from these cells does reveal a substantial presence of C-EBPβ in A4 cells (Fig. 7*C*). In these progenitor cells, C-EBPβ appears to be restricted to the cytoplasm in a hypophosphorylated and presumably inactive form. In contrast, in granulocyte-committed D+ cells most of the C-EBPβ present is localized in the nucleus as the slower migrating phosphorylated (active) form (Fig. 7*C*). Finally, although C-EBPδ was not detected in the nucleus (Fig. 7*D*) or cytoplasm of A4 cells (data not shown), this isoform is present in the nucleus of D+ cells (Fig. 7*D*) and migrates as a single enhancer binding form of about 33 kDa.

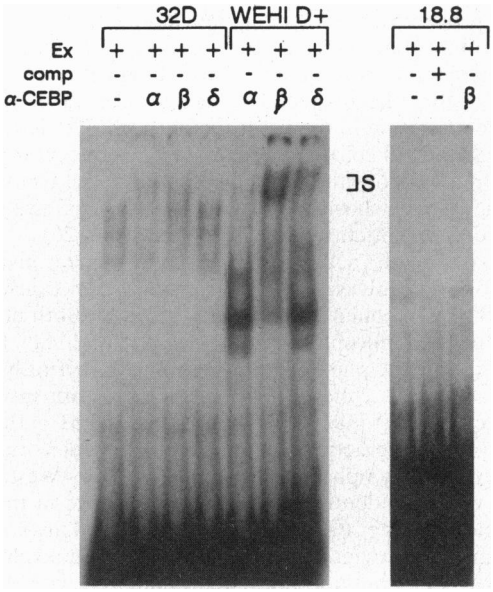

FIG. 5. FTII binds multiple isoforms of the C-EBP family in myeloid cells. DNA-binding assays were performed in the presence of labeled FTII and nuclear extracts from various cell types. Extracts were preincubated in the presence of anti-sera to C-EBPα, -β, and -δ for 1 hr before addition of probe. S, the position of supershifts.

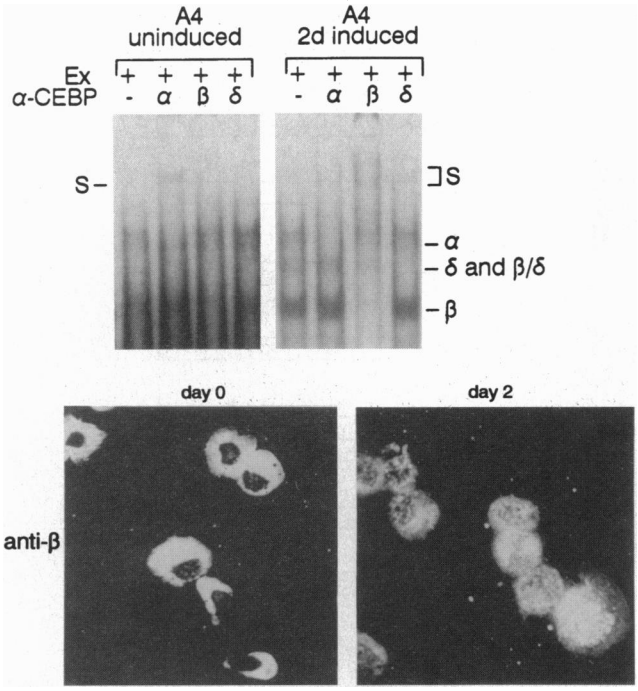

FIG. 6. (*Upper*) Differential regulation of the C-EBP family during granulopoiesis. DNA-binding assays were performed as above. Labeled FTII was mixed with nuclear extracts prepared from uninduced and day 2 granulocyte-induced A4 cells. The relative anti-sera are shown and their relevant supershifts are denoted by an S. (*Lower*) Immunoflourescence analysis and cellular localization of C-EBPβ proteins in uninduced (day 0) and day 2 granulocyte-induced A4 cells. Cytospins were stained with antibodies to C-EBPβ.

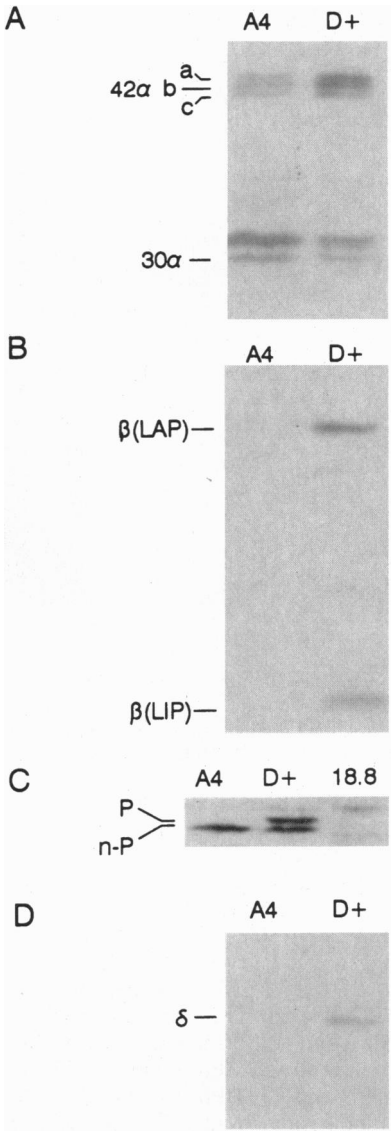

FIG. 7. Differential localization and activation status of the C-EBP family between granulocyte-committed cells and multipotential progenitor cells. Low bis-acrylamide Western blots were performed using anti-sera specific to C-EBPα, -β, and -δ as described above. (*A*, *B*, and *D*) Nuclear protein extracts from A4 and D+ cells probed sequentially with anti-sera to C-EBPα, -β, and -δ, respectively. (*C*) A total cell extract probed with C-EBPβ. Note the band of retarded mobility corresponding to phosphorylation of C-EBPβ in the nucleus; some nonspecific binding is also seen in 18.8 and D+ extracts. P, the slow migrating, phosphorylated form of C-EBPβ; h-P, the faster migrating hypo-phosphorylated form of the protein. These assignments were confirmed by electrophoresis of *in vivo*-phosphorylated C-EBP proteins (not shown).

DISCUSSION

The initial aim of the work described here was to examine the earliest events associated with MPO locus activation by identification of the transcription factors that bind to and activate the upstream MPO enhancer in myeloid cells compared with multipotential progenitor cells.

Regulation by Pu1. We found that Pu1 bound to the FTI sequence in both myeloid-committed cells and in multimyeloid progenitor cells. An oligonucleotide from an adjacent region to FT1 that also contains an ets-related binding motif could not significantly compete for binding (data not shown), suggesting that the distinct flanking sequences of the core motif are important in the control of binding. Pu1 is known to be expressed in B cells, myelomonocytes, and erythroid cells but not in T cells or other nonhematopoietic cell types, and in addition has been shown to regulate a number of myeloid and lymphoid differentiation-associated genes (24–26).

Cell-type specific activation of genes by many transcription factors often involves interactions with other transcription factors that are regulated by phosphorylation. Both nonphosphorylated and phosphorylated forms of Pu1 can bind to DNA, but only the phosphorylated form can recruit NF-EM5 to bind at the Igκ enhancer (NF-EM5 has no intrinsic DNA binding capability) (19). Phosphorylation of Pu1 is therefore critical for this interaction and appears to control its transcriptional activity in lymphoid cells. Interestingly, by Western blot analysis we have identified a distinct difference in the phosphorylation status of Pu1 between lymphoid and myeloid cells; in both myeloid progenitor (A4) and committed myeloid cells (D+), Pu1 was nonphosphorylated compared with its phosphorylated state in pre-B cells. Our results raise the intriguing possibility that phosphorylation of Pu1 may play a pivotal role in control of lineage-specific gene expression.

Regulation by C-EBP. The second and third elements of the enhancer contain a number of binding sites for the C-EBP class of transcription factors that contain a leucine zipper region flanked by a basic DNA binding domain (for review, see ref. 21). Members of the C-EBP family have been shown to regulate the terminal differentiation of adipocytes (for review, see ref. 27) and to show a different temporal expression pattern in differentiating myelomonocytes (28). Recently, targeted disruption of the C-EBPα and C-EBPβ genes has provided firm evidence for a role in energy homeostasis and bacterial killing, respectively (29, 30). Since each member of the C-EBP family can form homodimers or heterodimers with each other and also interact with other transcription factors such as the myb protoncogene (31), regulation of specific genes by C-EBP family members may be controlled by their affinity for a particular binding site or by relative protein interactions and posttranslational modifications. We have found at least four different binding sites for C-EBP family members in the MPO enhancer and have confirmed the presence and binding of different isoforms of C-EBP in myeloid cell and multipotential progenitor cell nuclear protein extracts. MPO-expressing cells have little of the C-EBPα isoform compared with that of C-EBPβ and -δ, whereas in multipotential cells only the α isoform is present. Since these different isoforms can compete for similar binding sites, it is conceivable that increased nuclear levels of C-EBPβ could displace C-EBPα isoforms and, in turn, C-EBPβ isoforms could be displaced by isoforms of C-EBPδ (32, 33). Such displacement could induce dramatic changes in the ratios of homo- and heterodimers and therefore mediate distinct regulatory functions.

Significantly, recent experiments show that C-EBPδ and Pu1 can physically cooperate to activate transcription in a synergistic manner (34).

At least two C-EBP isoforms α and β can bind DNA in a nonphosphorylated form, whereas the δ isoform requires phosphorylation for binding activity (35, 36). C-EBPβ is known to undergo cAMP-induced phosphorylation and translocation to the nucleus (37) and in addition phosphorylation of C-EBPβ (NF-M) has been shown to enhance transcription of myelomonocyte specific genes by revealing a previously concealed activation domain (38). In the present study, we have shown that in granulocyte precursor cells that express MPO (D+ cells), C-EBPβ is hyperphosphorylated and located in the nucleus as a DNA binding activity; in contrast, in more primitive myeloid progenitor cells that do not yet express the MPO gene, C-EBPβ is held in the cytoplasm in a hypophosphorylated, inactive form. Binding of G-CSF to its receptor on progenitor cells may therefore induce an activation cascade in which C-EBPβ and -δ become phosphorylated, are translocated to the nucleus, and participate in the transcriptional activation of the MPO gene and perhaps other granulocyte-specific genes. Recently, Friedman and colleagues (39, 40) have reported an enhancer element in the promoter region of the murine MPO gene that also contains several functional elements and binds members of another family of transcriptional activators, the PEBP2/CBF (polyoma virus enhancer-binding factor 2/core binding factor) family. It will be interesting to determine to what extent phosphorylation is required for interactions between PEBP2/CBF, the C-EBP family, and Pu1.

Perspective. In summary, our results implicate Pu1 and the C-EBP family of transcription factors in the regulation of the MPO gene in granulocytic cells. Pu1 is present as a MPO-enhancer binding activity in multipotential cells before granulocyte commitment and differentiation. Although Pu1 is phosphorylated in pre-B cells, it is present in a nonphosphorylated form in multipotential and granulocyte-committed cells. With regard to the C-EBP family, the transition from a primed state in progenitor cells to an active MPO locus in granulocyte-committed cells is associated with changes in the expression, phosphorylation, and cellular localization of the different C-EBP isoforms. The assembly of an active chromatin configuration at a lineage-specific enhancer, such as 5′HS4, before uni-lineage commitment is not unique to the MPO locus. We have previously shown that self-renewing multipotential progenitor cells partially activate lymphoid, erythroid, and monocytic programs before a commitment decision is taken (4, 11, 41). The molecular mechanisms that underlie the priming of these loci remain to be elucidated. GATA-1 has been implicated in β-globin locus control region function in erythroid cells; however, GATA-2 is the predominant form in uncommitted multipotent progenitor cells (42) in which the β-globin locus control region is already in an active chromatin configuration (41). By analogy to the MPO enhancer, it is tempting to speculate that erythroid differentiation and functional activation of the β-globin locus is accompanied by a displacement of GATA-2 protein by GATA-1. Differentiation-associated changes in factor occupancy at critical regulatory elements may therefore prove to be a common theme in the activation of lineage-specific loci.

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