Myeloid Expression of Cytochrome P450 4F3 Is Determined by a Lineage-specific Alternative Promoter

The cytochrome P450 4F3 (CYP4F3) gene encodes two functionally distinct enzymes that differ only by the selection of exon 4 (CYP4F3A) or exon 3 (CYP4F3B). CYP4F3A inactivates leukotriene B4, a reaction that has significance for controlling inflammation. CYP4F3B converts arachidonic acid to 20-hydroxyeicosatetraenoic acid, a potent activator of protein kinase C. We have previously shown that mRNAs coding for CYP4F3A and CYP4F3B are generated from distinct transcription start sites in neutrophils and liver. We therefore investigated mechanisms that regulate the cell-specific expression of these two isoforms. Initially, we analyzed the distribution of CYP4F3 in human leukocytes and determined a lineage-specific pattern of isoform expression. CYP4F3A is expressed in myeloid cells and is coordinate with myeloid differentiation markers such as CD11b and myeloperoxidase during development in the bone marrow. In contrast, CYP4F3B expression is restricted to a small population of CD3+ T lymphocytes. We identified distinct transcriptional features in myeloid, lymphoid, and hepatic cells that indicate the presence of multiple promoters in the CYP4F3 gene. The hepatic promoter depends on a cluster of hepatocyte nuclear factor sites 123–155 bp upstream of the initiator ATG codon. The myeloid promoter spans 400 bp in a region 468–872 bp upstream of the ATG codon; it is associated with clusters of CACGT sites and can be activated by ZEB-2, a factor primarily characterized as a transcriptional repressor in cells that include lymphocytes. ZEB-2 interacts with C-terminal binding protein and Smads, and this would provide opportunities for integrating environmental signals in myelopoiesis and inflammation.

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The ability of cells to attain differentiated stages in development and to alter their functional phenotype in inflammatory settings depends on lineage-specific and cytokine-dependent transcription factors that either activate or repress target genes. Distinct patterns of transcription factors are associated with the myeloid and lymphoid lineages in hematopoiesis (1). and with the induction of pro-inflammatory genes in host defense (2). Transcription factor activity is context-dependent; a repressor of one gene can activate a different gene associated with an opposing function or alternative cell lineage (3). This holds for the coordinated induction and repression of sets of genes that are functionally related. ZEB-1 (ΔEF1, Zfx1a) and ZEB-2 (SIP1, Zfx1b) are recently characterized two-handed zinc finger transcription factors (4, 5) that may function as both gene silencers and activators. ZEBs repress transcription of genes that include IL-2, CD4, and GATA-3 in lymphocytes (6–8), whereas their role in myeloid cells has not been determined. The C-terminal binding protein (CtBP)‡ is a corepressor for ZEBs and other factors but does not bind DNA directly (9). Recent evidence suggests that ZEBs can activate the vitamin D3 receptor gene in colon carcinoma cells (10).

Cytochrome P450 4F3 (CYP4F3) functions to control inflammation by inactivating leukotriene B4 (LTB4) with high specificity (11–13), but the mechanisms that regulate CYP4F3 gene expression are not known. LTB4 is a potent chemoattractant of myeloid cells (14–17) and has been implicated in the pathogenesis of multiple inflammatory diseases (18–21). LTB4 is synthesized from arachidonic acid by the sequential action of 5-lipoxygenase and leukotriene A4 hydrolase, and the chemotactant activity of LTB4 is mediated by a high affinity G protein-coupled receptor designated BLT1 (22). The major pathway for the catabolism and inactivation of LTB4 in human neutrophils involves microsomal ω-hydroxylation by CYP4F3 (11). This reaction generates 20-OH-LTB4, which can further inhibit LTB4 activities by down-regulating BLT1 (23). The ability of LTB4 to amplify an inflammatory response is, therefore, counterbalanced by the expression of CYP4F3.

Recently we cloned the CYP4F3 gene and demonstrated that alternative splicing generates isoforms that differ in functional properties and tissue distribution (24, 25). Selection of exon 4 generates the neutrophil isoform (CYP4F3A), which has a low Km for LTB4 of <1 µM. Selection of exon 3 instead of exon 4 generates an alternative isoform (CYP4F3B), which has a 44-fold lower efficiency of inactivating LTB4. CYP4F3B is expressed in liver and has a preference for arachidonic acid as a substrate, which it converts to 20-HETE (25). This has significance because 20-HETE is a potent bioactive mediator in certain tissues; it activates protein kinase C and has roles in regulating cell proliferation, vascular tone, and natriuresis (26). These opposing capacities of the CYP4F3 isoforms to generate an active mediator (20-HETE) or inactivate one (LTB4) allow for versatility of function but demand strict con-
trols. CYP4F3 transcription and alternative splicing must be regulated to ensure that the appropriate isoform is generated in the correct setting.

To understand the regulation of CYP4F3 expression, we examined its distribution in maturing populations of human bone marrow cells and identified the splicing pathways. CYP4F3A is expressed in myeloid cells; its expression is coordinate with known myeloid differentiation markers such as CD11b and also increases concomitantly with myeloperoxidase during development. In contrast, CYP4F3B is expressed in lymphocytes, and expression is restricted to a small population (~10%) of CD4+ T cells. We determined that the alternative pre-mRNA generated by a single site-specific splicing of CYP4F3A is expressed in myeloid cells, lymphoid cells, and liver. Surprisingly, activity of the myeloid-specific promoter could not be accounted for by known myeloid transcription factors including PU.1 and MZF-1, but it could be activated by ZEB-2 and CtBP.

The results suggest new roles for these proteins in myeloid transcription. ZEBs and CtBPs are regulated by activated Smads and NAD+, respectively, and therefore have the capacity to integrate various environmental signals during development or inflammation.

**EXPERIMENTAL PROCEDURES**

**Cells**—Human bone marrow was obtained from discarded filters used in the processing of normal donor marrow from transplantation at the Massachusetts General Hospital. Peripheral blood samples were collected from healthy donors according to established guidelines. Approval for the use of human samples was obtained from the Institutional Review Board of the Massachusetts General Hospital/Partners. Granulocytes and lymphocytes were separated by Ficoll-Hypaque (Amersham Biosciences) density gradient centrifugation. HL60 cells (human promyelocytic cell line) were maintained in RPMI containing 10% fetal bovine serum. The cells were treated with 1.3% Me2SO for 4 days to induce granulocytic differentiation before transfection experiments. HepG2 cells (human hepatoma-derived cell line) and COS 7 cells were maintained in Dulbecco's modified Eagle medium containing 10% fetal bovine serum.

**Flow Cytometry**—Total bone marrow mononuclear cells and peripheral blood granulocytes and lymphocytes were stained with allophycocyanin, percpycoerythrin, or fluorescein isothiocyanate-conjugated control IgGs or monoclonal antibodies directed to CD34, CD38, CD33, CD13, CD11b, CD15, CD14, CD8, and CD5 (BD Biosciences) to define hematopoietic cell subsets in a double or tri-color labeling. Surface-labeled samples were then fixed in 0.1% formaldehyde and permeabilized with 0.1% Triton X-100 to perform intracytoplasmic staining with anti-CYP4F3 antibody. Production, purification, and characterization of polyclonal rabbit antibodies against the C-terminal domain (amino acids 520) of CYP4F3, which recognize both isoforms (CYP4F3A and CYP4F3B) have been described previously (24). Intracellular staining was performed on unlabelled or surface-labeled cells after fixation and permeabilization. Samples were incubated with 5 μg/ml anti-CYP4F3 polyclonal antibody or 3 μg/ml control rabbit IgG for 30 min at room temperature and were then incubated with 1 μg/ml goat anti-rabbit phycoerythrin-conjugated or fluorescein isothiocyanate-conjugated polyclonal antibodies (Sigma). Tri- or four-color-labeled samples were then analyzed by flow cytometry using the FACScalibur instrument and the CellQuest program (BD Biosciences). The data were plotted as single-parameter histograms or bivariate dot plots with logarithmic amplification.

**RNA Isolation, Reverse Transcription, and Isoform-specific PCR**—Total RNA was isolated from cells using TRI Reagent (Sigma). Reverse transcription and first-strand CDNA synthesis was performed using the cDNA cycle kit (Invitrogen) with random primers and avian myeloblastosis virus reverse transcriptase. The cDNA was purified by phenolchloroform extraction and ethanol precipitation. CYP4F3A and CYP4F3B were isolated by isoform-specific PCR by using primers specific for ZEB-1 (forward, 5'-CTGAAGAGG-ACCAGAGGCAGC-3', reverse, 5'-CCCAAGCTCGTGCTACAGTGTC-3'), ZEB-2 (forward, 5'-CTGAGGAGCTGGTCTCCGCTT-3', reverse, 5'-GCTTCTGGGGATCATTGTTG-3'), or primers that would bind to CtBP-1 and CtBP-2 (forward, 5'-CCAGGAAGGGACCTGGAGAAGTC-3'; reverse, 5'-GACACCTGGGAGATCAGAGTGC-3') that were used in PCR reactions with conditions of 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min for 30 cycles.

**RESULTS**

**CYP4F3 Expression in Human Bone Marrow**—To understand the regulation of the CYP4F3 gene and its isoforms, we initially determined the expression of the CYP4F3 protein in different populations of developing hematopoietic cells. Non-
erythroid nucleated bone marrow cells were isolated from four individual donors and stained with affinity-purified fluorescent-labeled anti-CYP4F3 for analysis by flow cytometry. Analysis of total non-erythroid bone marrow cells indicated that 40–60% expressed CYP4F3 protein (Fig. 1A), with 30–40% exhibiting high and 10–20% exhibiting intermediate fluorescence. To identify cell populations for further analysis, bone marrow cells were next broadly separated into the subdivisions R1, R2, and R3 (Fig. 1B) based on the properties of size (forward scatter) and granularity (side scatter). These populations were analyzed for CYP4F3 expression using rabbit IgG as a negative control (Fig. 1C). A small but consistent population of cells (3%) demonstrated positive staining for CYP4F3 in R1 (pink), the region of low forward scatter, and low side scatter that includes lymphocytes and early progenitors. The region R2 (green) includes a mixed population of cycling progenitors, monocytes, and maturing myeloid cells, and 64% of these cells were positive for CYP4F3. This region was further separated into the subdivisions R4, R5, and R6 (Fig. 1D), and a progressive increase in CYP4F3 expression (49, 66, and 76% positivity, respectively) was observed with increasing granularity (maturity). Cells with the highest levels of CYP4F3 expression were concentrated in R3 (red), a region of low forward scatter and high side scatter that is composed primarily of mature granulocytes (Fig. 2C). Essentially all of these cells (99%) were positive. The results indicate that expression of CYP4F3 is concomitant with myeloid maturation.

We next compared the expression of CYP4F3 in bone marrow to that of known myeloid proteins using a panel of antibodies against surface markers for hematopoietic differentiation and maturation in conjunction with anti-CYP4F3 (Fig. 2). A representative experiment is shown in Fig. 2A. A high percentage of total bone marrow cells are double-positive for CYP4F3 and myeloid markers (for example, 48% CD33+/H11001, 37% CD11b+/H11001, and 32% CD15+/H11001 cells express CYP4F3). Less than 2% of cells are double-positive for CYP4F3 and lymphoid markers such as CD2, CD3, and CD8. Interestingly, this population of lymphocytes expressing CYP4F3 was consistently observed, and it is apparent that some of the cells have a high level of expression. The data presented for total bone marrow cells in Fig. 2A were quantified to determine the expression of each marker protein in the CYP4F3+ population (Fig. 2B). Most CYP4F3 positive cells (>90%) express myeloperoxidase (MPO) and CD33, and a high proportion of CYP4F3 positive cells express other myeloid markers including CD15 (72 ± 2%), CD38 (62% ± 2%), CD11b (54 ± 16%), CD13 (45 ± 12%), CD14 (15 ± 3%), and CD31 (14 ± 2%). In contrast, antigens associated with T and NK lymphocytes (CD2, CD3, CD8, and CD16) or hematopoietic progenitors (CD34) were only expressed in 0.7–2.5% of the CYP4F3+ cells.

An alternative method of evaluating the data is shown in Fig. 3A.
also expressed in a small population of CD34+ cells but indicate that it is positive for CYP4F3. Overall, the results confirm that CYP4F3 expression in CD33+ maturing myeloid cells, we compared CYP4F3 and MPO expression in developing myeloid cells. 

To determine whether CYP4F3 is differentially expressed in maturing myeloid cells, we compared CYP4F3 and MPO expression in CD33+ gated populations (Fig. 3). Cells that were negative or low for MPO also had low expression of CYP4F3, and CYP4F3 expression increased in parallel with MPO. This is consistent with the increases in CYP4F3 fluorescence observed as a function of cell granularity (Fig. 1) and confirms that CYP4F3 expression is strongly associated with myeloid differentiation.

Expression of CYP4F3 in Peripheral Blood Leukocytes—Flow cytometry was used to compare the expression level of CYP4F3 in different cell types in peripheral blood (Fig. 4). Granulocytes exhibit high fluorescence (Fig. 4A), whereas lymphocytes exhibit low fluorescence similar to negative control staining with IgG (Fig. 4B). When peripheral leukocytes were analyzed for myeloid and lymphoid markers that included CD15, CD14, CD3, and CD8, the results paralleled those in bone marrow in that essentially 100% of CD15+ and CD14+ cells but only ~10% of CD3+ and CD8+ cells co-expressed CYP4F3 (not shown). A HL60 promyelocytic leukemia cell line exhibited intermediate fluorescence (Fig. 4C). Analysis of blood from two patients with eosinophilia gave identical results; CYP4F3 was expressed at highest levels in the eosinophils followed by neutrophils and then monocytes (Fig. 4, D and E). Eosinophils samples were analyzed for CD16, CD45, and CD49D in addition to the markers listed above (not shown).

Alternative Promoters Regulate Tissue-specific Expression of CYP4F3—We have previously shown that functionally distinct splice forms of CYP4F3 exhibit a tissue-specific distribution, with CYP4F3A expressed in neutrophils, and CYP4F3B expressed in liver (24, 25). We suggested that the 5'UTR of CYP4F3 transcripts is also tissue-specific and might indicate the use of alternative promoters in different cell types (24). To further investigate this possibility, RNA samples from selected cell populations were analyzed by 5'-RACE to determine the sequence of the 5'-UTR and to identify the transcription start sites (Fig. 5A). Similar results were obtained in peripheral blood granulocytes, bone marrow myeloid cells isolated at early (BM-1, CD11b+ CD14-) or later (BM-2, CD11b+ CD14+) stages of development, and also HL60 cells; a single transcription initiation site was identified 519 bp upstream of the ATG initiation codon (start site A). The 5'-UTR requires splicing to assume its location upstream of the ATG codon in mature myeloid transcripts, and the only variation observed relates to selection of the 5'-splice donor site. Two alternative splice junctions are used in bone marrow myeloid cells, apparently with similar efficiency, to generate 5'-UTRs of 40 or 34 bp. The 40-bp 5'-UTR was the only form observed in peripheral blood granulocytes. In HepG2 cells, transcription is initiated at a site located 71 bp upstream of the initiation codon (start site B).

Fig. 2. Immunophenotypic characterization of CYP4F3 positive cells. A, each panel shows a representative double-labeling experiment for CYP4F3 and a selected marker and indicates % double-positive cells in total bone marrow. The thresholds of positivity were established by staining with negative control IgG. B, the percentage of cells expressing each marker in the CYP4F3 positive subpopulation was determined as an average of four experiments (error bars indicate S.D.). C, percentage of cells expressing CYP4F3 in populations positive for specific myeloid (CD15, CD11b, CD14), lymphoid (CD16, CD8, CD3), or stem cell (CD34) markers (error bars indicate S.D., n = 4).

Fig. 3. Correlation of CYP4F3 and MPO expression in developing myeloid cells. A, CD33+ gated bone marrow cells (stained with anti-CD33-allophycocyanin) were stained with anti-MPO-phycocerythrin and analyzed by flow cytometry. B, cells with low (R1), intermediate (R2), or high (R3) MPO expression were analyzed for CYP4F3 immunofluorescence using fluorescein isothiocyanate-conjugated secondary antibodies.
The Myeloid Promoter Is Activated by ZEB-2 and CtBP—We initially sought to identify a role for known myeloid transcription factors in the regulation of CYP4F3 gene expression. Potential binding sites were predicted by inspection of the DNA sequence of the myeloid promoter (region −872 to −468) and are summarized in Table I. These include putative sites for the factors MZF-1 (based on the core sequence GGXXA, positions −602, −538, and −515) and PU.1 (permissible variations of the GAGGAA core sequence, positions −575 and −518). Surprisingly, mutations to these sites did not reduce activity (Table I). We then focused on the region in the 5′-UTR between −500 and −468, which is an active element of the myeloid promoter. This region contains a putative ZEB target site; ZEB-1 (5EFP) and ZEB-2 (SIP1) are two-handed zinc finger transcription factors that bind to bipartite CACCTG sequences with a variable orientation and spacing of nucleotides (4). Two inverted CACCT sites (AGGTG)(121,900),(745,917) with a spacing of 11 bp are located between positions −488 and −468, a location that exactly matches the two splice junctions (Fig. 6A). Furthermore, a similar bipartite motif is observed further upstream between positions −804 and −783, a location that is within a region of the promoter required for activity in HL60 cells (compare constructs A5 and A6 in Fig. 5C).

The effect of ZEB expression on CYP4F3 promoter activity was measured in COS 7 cells (Fig. 6B). The cells were cotransfected with a ZEB expression vector and construct A6, which contains the myeloid promoter and both bipartite ZEB sites at positions −488 and −804. A 12-fold induction of activity was observed if an expression vector for CtBP was included. No induction of luciferase activity was observed in the absence of CtBP. The response was dependent on the amount of ZEB expression vector used for transfection and decreased in an approximately linear fashion from 1 to 0.25 μg vector. No significant differences were observed in the response to expression vectors for ZEB-1 or ZEB-2. Mutations to the ZEB sites were investigated using constructs A5 and A3, which contain one bipartite site at position −488, or −804, respectively. ZEB and CtBP induce a 6-fold increase in activity of construct A5, and this is abolished by deleting the region −500 to −468 (mut1). ZEB and CtBP induce a 15-fold increase in activity of construct A3, and this can be reduced to 2-fold by a site-specific mutation that converts AGGTG to CCGTG at position −804 (mut2). Construct A3 does not contain any splice junctions, and the effect of mut2 on transcription is, therefore, clearly separable from any effects on splicing. The activity of these mutants in HL60 cells is shown in Table I. Deletion of the ZEB site in exon 1 (Δ−500 to −468) results in a 71% decrease in activity of construct A5, and the site-specific mutation at position −804 results in an 88% decrease in activity of construct A3.

The expression of ZEB-1 and ZEB-2 in myeloid cells was analyzed by isomark-specific reverse transcription-PCR (Fig. 7A). Bone marrow CD11b+ cells, peripheral blood granulocytes, and HL60 cells express ZEB-2 but not ZEB-1. HepG2 cells and CD3+ T cells preferentially express ZEB-1, and CtBP was detected in all samples. ZEB-1 and ZEB-2 are highly related and bind to identical DNA sequences, but they may have different transcriptional activity due to differences in their strength of interaction with other proteins (5). A gel retardation assay was then used to demonstrate binding of Myc-tagged ZEB-2 to the CYP4F3 promoter (Fig. 7B). A 32P-labeled oligonucleotide probe corresponding to the region between −812 and −775 of the promoter forms a binding complex with ZEB-2 (Fig. 7B, lane 2, the band indicated by the arrow). Binding is abolished by the addition of a 100-fold molar excess of unlabeled WT oligonucleotide (lane 3) but not by a mutant oligonucleotide which has the AGGTG sequences converted to
CCCGA (lane 4). Furthermore, the band disappears after the addition of anti-Myc antibody (lane 5) but not anti-lamin antibody (lane 6). The location of the supershifted band is masked by the presence of a larger complex, but the signal intensity in the region of the larger complex increases after the addition of anti-Myc antibody (lane 5), not anti-lamin antibody (lane 6).
sites have low or minimal effect on the activity of promoter constructs (Table 1), and the alternative promoters in the CYP4F3 gene may, therefore, belong to a class that does not depend on a TATA box. The hepatic and myeloid promoters overlap between positions −812 and −775 of the CYP4F3 promoter was incubated without (lane 1, control) or with (lane 2) nuclear extract from COS 7 cells transfected with Myc-tagged ZEB-2. The following additions were made to DNA binding assays containing nuclear extract: excess unlabeled WT oligonucleotide (lane 3); excess unlabeled mutant oligonucleotide with the CACCT sites destroyed (lane 4); anti-Myc monoclonal antibody (lane 5); anti-Myc monoclonal antibody (lane 6). The arrow indicates the position of a binding complex, which is competed with WT oligonucleotide and supershifted with anti-Myc antibody.

Peripheral blood CD3+ T lymphocytes were isolated to high purity (>99%) by flow cytometry, and 5'-RACE was performed on RNA preparations to determine the transcription start site of CYP4F3 in these cells. The sequences of 10 5'-RACE clones were identical and identify a 19-bp 5'-UTR with the sequence TACCTAGTGGCTGTACAGG. This indicates a novel transcription start site (designated start site C) located 219 bp upstream of the ATG initiation codon, intermediate between start sites A and B (Fig. 8A). It is separated from the myeloid transcription start site A by 300 bp, raising the possibility that distinct promoter elements are recruited to enable expression in lymphoid cells. This was not characterized in detail. Transcription initiation from start site C requires splicing to link the 5'-UTR to the ATG initiation codon and is independent of transcription initiation from start site B.

The splicing pathways of CYP4F3 in myeloid cells, liver, and T cells are shown schematically in Fig. 8B. Selection of exon 4 is the critical event that determines CYP4F3 function in myeloid cells. Recently we cloned the CYP4F3 gene (24) and showed that a unique feature of exon 4 is a 25-bp repeat that duplicates
Concomitant with increased cell granularity and acquisition of myeloid differentiation markers such as myeloperoxidase. In peripheral blood, CYP4F3 is expressed at highest levels in eosinophils followed by neutrophils and then monocytes (Fig. 4).

The presence of CYP4F3 in eosinophils and its strong association with myeloid differentiation in bone marrow have not previously been shown but are consistent with its functional involvement with LTβ regulates. LTβ is a chemoattractant for human eosinophils (17), and eosinophils isolated from patients with hypereosinophilic syndromes express abundant cytosolic phospholipase A₂, 5-lipoxygenase, and BLT1 (29). Interestingly, an average of 20% CD34+ progenitor cells in the bone marrow exhibit CYP4F3 reactivity (Fig. 2C), and this may be due to populations of early myeloid-committed cells that have been identified in previous studies by coexpression of CD34 and CD11b (30). An early onset of CYP4F3 expression in bone marrow would be consistent with a role in maintaining low basal levels of LTβ in addition to regulating LTβ activity during inflammation. CYP4F3 may protect against an inappropriate amplification of LTβ production as developing myeloid cells express BLT1 and LTβ1 biosynthetic enzymes.

We recently cloned the CYP4F3 gene and postulated that alternative promoters regulate transcription in liver and neutrophils because distinct 5′-UTRs are generated in each location (24). Many cytochrome P450s including CYP4F3 are expressed in liver, whereas expression in myeloid cells is unusual. The existence of an alternative promoter for myeloid expression is now confirmed using HL60 cells as a model system. Overlapping but distinct promoter regions were identified in HL60 and HepG2 cells transfected with luciferase reporter constructs containing 5′-flanking regions of the CYP4F3 gene (Fig. 5). The myeloid promoter identified in HL60 cells extends over a relatively short region (350 bp) upstream of transcription start site A (the myeloid start site), requires exon 1 (50 bp immediately downstream of the start site) for full activity, and a TATA box is not essential. Similar features have been observed in promoters for other myeloid-specific genes (31). The presence of alternative promoters represents another unusual feature of the CYP4F3 gene and is comparable with the situation described for CYP19 (aromatase) expression that has been intensively investigated (32). Transcription of CYP19 is regulated by different promoters in different tissues and results in transcripts with different 5′-UTRs, although the protein encoded by these transcripts is always the same.

The myeloid promoter of CYP4F3 includes potential sites for traditional myeloid transcription factors such as MZF-1 and PU.1, but mutations to these sites did not reduce activity in differentiated HL60 cells. An active region of exon 1 contains a bipartite ZEB site consisting of two inverted CACCT sequences separated by 11 bp (Fig. 6A), and deletion of this region reduces transcriptional activity by 71% (Table I). Interestingly, the location of these inverted CACCT sequences corresponds to the location of two 5′-splice donor sites in the pre-mRNA. A similar bipartite ZEB site is located at position −804, 285 bp upstream of the transcription start site, in another active region of the promoter. Mutagenesis of the site at −804 reduces transcriptional activity by 88% (Table I), and this effect is clearly independent of any potential interference with splicing. Two related ZEB genes have been identified, and both are expressed in hematopoietic cells; ZEB-1 (ξΕF1) is present in T cells, and ZEB-2 (SIP1) is present in B cells (5). Previous studies have focused on the role of ZEB genes in lymphocytes and have not specifically investigated myeloid cells. We used isoform-specific PCR to determine that ZEB-2, but not ZEB-1, is expressed in peripheral blood granulocytes and developing myeloid cells in

**DISCUSSION**

The CYP4F3 enzyme functions to inactivate nanomolar concentrations of LTB₄ for chemotaxis by catalyzing its α-hydroxylation (11-13). 20-OH LTB₄ binds to BLT1 with the same high affinity as LTB₄ and inhibits responses to LTB₄ by downregulating the receptor (23). Determining the distribution of CYP4F3 expression should, therefore, identify cells that participate in the modulation of LTβ function. An understanding of mechanisms that regulate CYP4F3 gene expression might elucidate pathways that control LTβ-mediated inflammation. We used flow cytometry to analyze the distribution of CYP4F3 in human bone marrow cells and observed an expression pattern that was closely correlated with developing myeloid cell populations (Figs. 1–3). Increases in CYP4F3 fluorescence were
and observed a 10–CYP4F3 activity is more sensitive to context in the
by mutagenesis or deletion of the ZEB sites. Activation of the
above manner (39). It is possible that ZEB/CtBP represses tran-
flammation. It is well documented that CtBP acts as a core-
mechanisms of
5′-splice donor sequence of exon 4 (Fig. 8C), and this is similar
to exon 1 (the myeloid 5′-UTR), where two alternative 5′-splice
donor sequences correspond with the location of a bipartite
ZEB site. The ZEB transcription factors have a number of
unusual features; they can act as transcriptional repressors or
activators (6, 7, 8, 10); they interact with multiple proteins
including CtBP and Smads (33, 34); they bind with two zinc
finger regions to two target sites (4); they can act over long
distances and may serve as bridging molecules (44). It is inter-
esting to speculate that they might also play a role in splicing.
There is growing evidence that transcription and splicing are
coupled in many genes (45), and this would have particular
relevance to myeloid CYP4F3 expression and its regulation in
oxidative stress and inflammation.
Expression of the CYP4F3 gene involves alternative choices at
the level of transcription initiation, transcription termina-
tion, and splicing. Alternative splicing of CYP4F3 represents
the first recorded example of functionally different enzymes
being encoded within a single CYP gene (25); substrate speci-
ficity is determined by selection of exon 4 (CYP4F3A) or exon 3
(CYP4F3B). 3′-RACE analysis has identified two transcription
termination signals that generate transcripts of 5.034 and
2.339 kb (24, 46), although it is not yet clear whether these
transcripts are differentially distributed. In this report we
show that alternative promoters regulate CYP4F3 expression
in myeloid and hepatic cells. A distinct transcription start site
in lymphoid cells suggests that there are at least three tissue-
specific promoters in the gene. Regulation of CYP4F3 gene
expression is clearly complex and must involve coordinated
actions at all levels of mRNA processing to generate the correct
isoform in specific locations.

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