Myeloid ELF1-like Factor Is a Potent Activator of Interleukin-8 Expression in Hematopoietic Cells*

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Myeloid ELF1-like factor (MEF), also known as ELF4, is a member of the ETS family of transcription factors which is expressed in hematopoietic cells. MEF-deficient mice have defects in natural killer cell and natural killer T cell development, suggesting a role for MEF in regulating innate immunity. MEF also functions in myeloid cells, where it can transactivate target genes. To identify MEF target genes in a “myeloid” environment, we created an inducible expression system and used oligonucleotide microarrays to examine the transcript profile of HEL cells after induction of MEF expression. Sixteen genes were reproducibly turned on or off more than 2-fold, 8 h after induction of MEF expression, and we examined one of the genes, interleukin-8 (IL-8), in greater detail. IL-8 is a CXC chemokine involved in neutrophil chemotraction, angiogenesis, and stem cell mobilization. It is expressed by several tumor types, and its expression is regulated primarily transcriptionally. The IL-8 promoter contains three ETS binding sites, and we identified the specific site that binds MEF and is required for MEF responsiveness. MEF, but not the closely related ETS factors PEA3, ETS1, ETS2, ELF1, or PU.1, strongly activates the IL-8 promoter. MEF overexpression is sufficient to induce IL-8 protein expression, and reduction in MEF expression (using RNA interference) results in decreased IL-8 levels. These data demonstrate that MEF is an important regulator of IL-8 expression.

Myeloid ELF1-like factor (MEF), also known as ELF4, is a member of the ETS family of transcriptional regulators and was originally isolated from a myeloid hematopoietic cell line (1). ETS proteins generally regulate transcription in coordination with other transcription factors, binding the core ETS binding sequence GGAA, via their ETS domain. Because this sequence occurs frequently in genomic DNA, the identification of true ETS target genes has been difficult. Furthermore, the significant homology in the ETS domains of these proteins makes it daunting to determine which ETS factor regulates a particular gene in vivo. Although cytokine and cytokine receptor genes and many other genes have been proposed as targets of various ETS factors (for review, see Ref. 2), their validation as in vivo targets has rarely been performed.

Microarrays have been used extensively to profile the transcripts expressed in tumor or normal tissue, both as a way to define their biology and also to determine which genes serve as determinants for their classification. We and others have used microarrays to identify genes regulated by particular transcription factors such as C/EBPα (3), c-Myc (4), WT-1 (5), and BRCA-1 (6), among others. These studies have identified target genes not previously described and have failed to find gene targets previously identified by reporter gene assays. Thus, subsequent validation of direct (and indirect) targets will generally require the use of multiple additional approaches to confirm the results.

MEF is a member of the Drosophila E74 subfamily of ETS proteins which includes the mammalian NERF and ELF1 proteins. We have shown that MEF regulates cytokine gene expression, including GM-CSF and IL-3 (1). Other ETS factors have been shown to regulate the expression of chemokines, such as RANTES (regulated on activation normal T cell expressed and secreted) (7) and PF4 (8), and cell surface receptors including the M-CSF receptor (9), G- and GM-CSF receptors (10, 11) and CXCR1 (12). Although MEF and ELF1 are highly homologous, especially in their ETS domain, and they bind to the same DNA recognition sequence, MEF appears to be a more potent transactivator of promoter function (1). MEF also is required for perforin expression by NK cells but not activated cytotoxic T lymphocytes (34).

To identify MEF targets in hematopoietic cells, we established an inducible expression system in HEL cells, which have no detectable MEF mRNA or protein. We identified a number of MEF target genes using Affymetrix microarrays and focused primarily on IL-8, a member of the chemokine family with pleiotropic functions, including roles in stem cell mobilization (13), angiogenesis, and chemotaxis (14). The promoter of IL-8 has been identified previously, and both nuclear factor-κB and c-Jun have been shown to regulate its activity (15). IL-8 is expressed by a variety of tumor types and is inducibly expressed in several myeloid leukemia cell lines after 12-O-tetradecanoylphorbol-13-acetate stimulation (16). PEA3 has been suggested to be the ETS factor that controls IL-8 expression, although direct effects of PEA3 on IL-8 promoter function were not assessed (17). A recent study showed that MEF and ETS2 antagonistically regulate IL-8 expression in a lung carcinoma cell line, with MEF acting as a repressor (18). We show that MEF strongly activates the IL-8 promoter in hematopoietic and nonhematopoietic cells and that overexpression of MEF is sufficient to increase significantly the level of IL-8 protein expression. Furthermore, knock-down of MEF expression in cells that express both MEF and IL-8 by RNA interference, decreases...
MEF Regulates IL-8 Expression

EXPERIMENTAL PROCEDURES

Generation of Cells with Inducible MEF Expression—HEL cells and NB4–306 (a subclone of NB4, which is ATRA-resistant) cells were maintained in RPMI 1640 medium and COS-7 cells in Dulbecco’s modified Eagle’s medium, all supplemented with 10% fetal calf serum, penicillin, streptomycin, and glutamine. HEL cells were electroporated (using a Gene Pulser (Bio-Rad) set at 250 mV, 960 microfarads) with the pVgRXR plasmid (Invitrogen), which expresses a fusion of the edysone receptor and the X-box containing transactivation domain and the Erg box. Stably transfected clones were then selected in 50 μg/ml Zeocin (Invitrogen). These cells were electroporated with the pIND-MEF vector (which can express MEF in response to the active edysone receptor fusion protein), and clones were selected in 100 μg/ml hygromycin to isolate double transfected cells. To induce MEF expression, HEL/IND-MEF cells were incubated with the synthetic edysone analog, ponasterone A (Invitrogen), at a final concentration of 5 μM for 4, 8, 12, and 24 h. MEF levels were assessed by Western blot analysis using a 1:1,000 dilution of the MEF polyclonal antiserum (which is described elsewhere (19)).

Oligonucleotide Array-based Expression Profiling—Total RNA was isolated from MEF- or pIND-MEF cells after an 8-h incubation with 5 μM ponasterone A, using Trizol (Invitrogen) followed by RNeasy purification (Qiagen). The RNA was amplified, labeled, and hybridized to oligonucleotide arrays, as described elsewhere (20, 21), using the HuGeneFL Affymetrix GeneChip (Affymetrix, Santa Clara, CA) containing probe sets for 6,800 genes and expressed sequence tags. The results were analyzed with Affymetrix microarray suite (MAS 5.0). Only genes showing greater than 2-fold induction or repression, called “present” in the induced sample and called “induced” or “repressed” at a p < 0.001 by MAS 5.0, compared with base-line expression in HEL/VgRXR cells by MAS 5.0, compared with base-line expression in HEL/VgRXR cells. Samples were boiled, separated by SDS-PAGE, and transferred to Hybond-ECL membranes (Amersham Biosciences) for blotting with rabbit polyclonal anti-MEF antisera (previously generated (10)) using goat anti-rabbit horseradish peroxidase (Amersham Biosciences) as the secondary antibody. Blots were developed using the SuperSignal West Dura Extended Duration Substrate (Pierce).

Electrophoretic Mobility Shift Assays (EMSA)—MEF protein and PE3A protein were generated by in vitro transcription/translation using the TNT-coupled reticulocyte lysate system (Promega). 2 μl of in vitro translated MEF (or PE3A) was incubated with 1 ng of the indicated probes end-labeled with [γ-32P]ATP using T4 polynucleotide kinase (Invitrogen). Samples were electrophoresed using 6% polyacrylamide gels in 0.5× TBE. The specificity of binding was determined using a 100-fold excess of unlabeled probe as competitor. The complementary oligonucleotides used for the EMSA analysis are as follows: EBS2/3, 5′-ATAGAAACAAATGAAAGCTGTGAGCTACGG; EBS1, 5′-GATCAGCACGCGTAGCAGACCCC; EBS3, 5′-CCTGAGTCATCACACTgtCTATTTGTTCC; and for EBS1, 5′-ATAAGGAACAAATGAAAGCTGTGACTCAGG; EBS2, 5′-CTCTGACATAATG and 5′-CATTATGTCAGAGGAAATgtCACGTA-

Measurement of IL-8 Protein—Conditioned medium was collected from HEL/VgRXR and HEL/Ind-MEF cells at various time points after stimulation with ponasterone A and assayed using an IL-8 Quantikine kit (R&D Systems, Minneapolis, MN) per the manufacturer’s directions.

Real Time PCR Assays—One μg of total RNA was processed to cDNA by reverse transcription with Superscript II (Invitrogen) and an oligo(dT) primer, according to the manufacturer’s protocol. All PCR primers and TaqMan probes were purchased from Applied Biosystems (Sunnyvale, CA). Samples were processed using the Chromo4 real-time PCR detection system (Applied Biosystems); the thermal cycling conditions were 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Hypoxanthine-guanine phosphoribosyltransferase amplification was used to normalize the expression data for IL-8. Primers for amplifying the IL-8 and hypoxanthine-guanine phosphoribosyltransferase cDNA were obtained from Applied Biosystems.

Chromatin Immunoprecipitation—2 × 10^6 NB4–306 or HEL cells were processed using the chromatin immunoprecipitation assay kit (Upstate Biotechnology, Lake Placid, NY) according to the manufacturer’s instructions. Briefly, asynchronously growing NB4–306 or HEL cells were cross-linked with 1% formaldehyde for 10 min, and the reaction was stopped by the addition of glycine. The cells were washed in Tris-buffered saline and harvested in SDS buffer. After centrifugation, the cells were resuspended in immunoprecipitation buffer and sonicated (Fisher Scientific, model 500) to generate DNA fragments and purified by the manufacturer. The siRNA sequence selected for EBS3 is 5′-GGAAAGCAGGAATGTTGTTCTTC-3′ (antisense); 5′-GGCUACGUCCAGGAGCACC-3′ (sense). 200 pmol of siRNA was annealed and electroporated into the HindIII/XhoI site of the pIND vector to generate pIND-MEF. The 5′-region (−165 to +58) of the human IL-8 promoter was generated by PCR using genomic DNA as a template. The 5′-primer contained an XhoI site to facilitate cloning (5′-CCTGGACG- CATACCTCGATTTGGAAGGACACG; 3′-PCR primer contained an HindIII site (5′-GGCCTCTTGCTGTAAGCCTT). The PCR product was then digested with XhoI and HindIII and subcloned into pGL3Basic (to create pGL3-Basic-lS). To mutate ETS binding sites in this construct, the QuikChange mutation kit (Promega, Madison, WI) was used with the following primers (the mutated bp are in lowercase and underlined): for EBS1, 5′-CATACTGTTGCAAACCATGATGATT- TCTCCTGCTAATGT and 5′-CATTATGTCAGAGGAAATgTCGATTA- TTGACACTTGATT for EBS2, 5′-GCTGGGATCTGATGATGATGATGATGCT- AGG and 5′-CCTGAGTCTTCGTAAGCTTTTGC; and for EBS3, 5′-CCTGCAATTGATAACGACAATTGGAAGTGTG and 5′-CACACTTCCATTGTCGTCTACAAATCAGGAC. The DNA sequence of all wild type and mutated IL-8 promoter constructs was confirmed. PE3A was cloned by reverse transcription PCR from K562 cells using the Superscript one-step reverse transcription PCR system (Invitrogen) and the following primers: 5′-ATGAGGGAGGAGGATAGAAGGC and 5′-ATG- GAGGCGGAGGATAGAAGGC. The PE3A cDNA insert was sequenced (anti-sense; PE3A, Santa Cruz, SC-113, mouse monoclonal IgG1). The ETS1 and ETS2 plasmids were generous gift from Dr. Dennis Watson. The ELF1 cDNA was kindly provided by Craig Thompson and the PU.1 cDNA by Robert Heimberg. Expression from each of the expression constructs was confirmed by Western blot analysis of transiently transfected COS-7 cell extracts (data not shown).

Luciferase Reporter Assays—5 μg of either the pCMV5 cytomegalovirus promoter-based expression vector alone or pCMV5 containing the MEF, ETS1, ETS2, PU.1, ELF1, or PE3A cDNA (1) was used to cotransfect COS-7 cells using the calcium phosphate precipitation method together with 1 μg of pGL3-Basic-lS and 10 ng of pRL-CMV control plasmid, used to control for transfection efficiency. HEL cells were electroporated in RPMI with 10% fetal calf serum without antibiotics at 250 mV, 960 microfarads with 10 μg of reporter plasmid, 10 μg of expression vector, and 100 ng of pRL-CMV. Cell lysates were prepared 24 h after transfection using the dualluciferase assay system according to the manufacturer’s protocol (Promega).

Western Blot Analysis—Total cell lysates were prepared from COS-7 cells or HEL cells using radiolucent precipitation assay buffer, and protein concentrations were determined using Bio-Rad protein assay reagent. Samples were boiled, separated by SDS-PAGE, and transferred to Hybond-ECL membranes (Amersham Biosciences) for blotting with rabbit polyclonal anti-MEF antisera (previously generated (10)) using goat anti-rabbit horseradish peroxidase (Amersham Biosciences) as the secondary antibody. Blots were developed using the SuperSignal West Dura Extended Duration Substrate (Pierce).

RESULTS

MEF Target Gene Identification Using Microarrays—We used high density oligonucleotide microarrays to search for
genes whose expression is altered after the inducible expression of MEF in HEL human erythroleukemia cells (Fig. 1A). In these cells with tightly regulated, ponasterone A-inducible MEF expression, MEF protein is not present in the absence of ponasterone A but is detectable by Western blot as early as 2 h postinduction (Fig. 1B). To identify direct transcriptional targets, cells were grown in the presence of ponasterone A for 8 h, at which time total RNA was isolated and used to interrogate oligonucleotide arrays representing 6800 known genes and expressed sequence tags. Expression profiles in cells expressing MEF were compared with ponasterone A-stimulated HEL cells that contain the RXR/EcR heterodimer alone and do not inducibly express MEF (data not shown).

Using strict criteria, 13 genes were up-regulated greater than 2-fold, and three genes were down-regulated (Table I). If the -fold change threshold is lowered to 1.5 times, the list was expanded to 39 genes that were up-regulated and 14 genes that were down-regulated (data not shown). The most highly induced gene was IL-8 (which increased an average of 11.7-fold). Other genes induced by MEF included another chemokine, CXCL2 (gro-beta) and several other genes important to hematopoietic function including c-kit, MDR1, and phosphatidylinositol 3 kinase.

**MEF Regulates the IL-8 Promoter**—Given the magnitude of IL-8 mRNA induction by MEF, we sought to determine whether the IL-8 promoter might constitute a direct transcriptional target of MEF. We generated a reporter gene construct containing the /H11002 /H11001 region of the IL-8 promoter, which contains three potential EBSs upstream of the luciferase gene. Cotransfection assays in both COS-7 cells and HEL cells showed that MEF activates the IL-8 promoter (72-fold in COS-7 cells and 14-fold in HEL cells). In contrast, none of the related ETS transcription factors ELF1, PEA3, ETS1, ETS2, or PU.1 transactivates this construct more than 2-fold (Fig. 2, A and B). Mutation of two of the three potential ETS binding sites in the IL-8 promoter (EBS2 and EBS3), did not alter its MEF responsiveness or its basal activity (Fig. 2C). However, MEF responsiveness was lost when the most 3′-ETS binding site (EBS1) was mutated. Mutations in the individual EBSs did not significantly affect the basal IL-8 promoter activity (mEBS1, 1.6-fold; mEBS2, 1.2-fold, mEBS3, 1.3-fold relative to the construct with three wild type EBSs).

To determine whether the EBS1 ETS binding sequence was responding directly to the expression of MEF, we performed EMSAs to examine the binding of MEF (or PEA3) to EBS1, EBS2, and EBS3. Sequence-specific binding of MEF to the radiolabeled EBS1 probe was observed (Fig. 3A) which was competed by the wild type EBS1 oligonucleotide but not by a mutant EBS1 (mEBS1) or the EBS2/3 oligonucleotide that contains the other two ETS binding sites. MEF does not bind to EBS2/3 (Fig. 3B, lane 9). However we confirmed the work of Iguchi et al. (17) who showed that PEA3 binds to the 5′-AGGAAG-3′ sequence within EBS2 (Fig. 3B, lane 11). PEA3 also binds to EBS1 (Fig. 3B, lane 5); however, the IL-8 pro-

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**Fig. 1.** Strategy utilized for the identification of MEF target genes in HEL cells. **A**, expression profiling of HEL cells was performed either in absence of inducible MEF expression. **B**, Western blot analysis using protein extracts from HEL/IND-MEF cells induced with 5 µM ponasterone A for the indicated number of hours blotted with anti-MEF polyclonal rabbit antiserum. The size of molecular mass markers is indicated in kDa.

**Fig. 2.** MEF activates the IL-8 promoter via a specific EBS. COS-7 cells (A) or HEL cells (B) were transiently transfected with an IL-8 promoter luciferase reporter plasmid and the indicated expression vector. **C**, COS-7 cells were transiently transfected with either the wild type IL-8 promoter reporter plasmid (WT) or reporter plasmids containing single EBS mutations and the indicated expression vector. Data are expressed relative to the pCMV5 plasmid alone and are shown as the mean ± S.D. Cells cotransfected with pRL-CMV served as a control; luciferase activity was normalized to Renilla luciferase activity in each sample. The data shown are the average of three independent experiments.
moter is not activated by PEA3 expression in COS-7 cells.

MEF Is Bound to the IL-8 Promoter in Vivo—To establish whether MEF is bound to the IL-8 promoter in vivo, chromatin immunoprecipitation assays were performed using anti-MEF antiserum. Chemical cross-linking of chromatin from NB4–306 cells, which are known to express constitutively both MEF and IL-8 (16), was followed by immunoprecipitation with the indicated antibodies (Fig. 3C). Using PCR to amplify the −165 to +44 portion of the IL-8 promoter, we found MEF bound to this region of DNA in the basal state (lane 4). Using antibodies against other ETS factors that are expressed in NB4–306 cells but were not able to activate the IL-8 reporter in cotransfection studies, we found that neither ELF1 (lane 6), PEA3 (lane 7), PU.1 (lane 8), nor ETS2 (lane 9) was bound to the IL-8 promoter in NB4–306 cells. Of note, ETS1 was not detectable by Western blot in NB4–306 cells. In HEL cells, which do not express MEF, we could not immunoprecipitate the IL-8 promoter with anti-MEF antibodies (lane 5). Anti-acetyl-histone H3 (lane 4) and anti-c-Jun (lane 6) antibodies did detect binding of these proteins to the IL-8 promoter, as expected.

Table 1

| Name                        | Symbol | Function       | GenBank    | Fold change |
|-----------------------------|--------|----------------|------------|-------------|
| **Increased**               |        |                |            |             |
| IL-8                        | IL-8   | Chemokine      | Y00787     | 11.7        |
| Metallothione-IG            | MTG1G  | Heavy metal binding | J03910    | 8.5         |
| L-Histidine decarboxylase   | HDC    | Metabolism     | D16583     | 5.2         |
| Down syndrome critical region gene 1 | DSCR1  | Signaling      | U28833     | 3.5         |
| CXCL2                       | CXCL2  | Chemokine      | M57731     | 3.0         |
| TAF7                        | TAF7   | Transcription  | U18962     | 3.0         |
| ATP-binding cassette, subfamily B (MDR/TAP), member 1 | ABCB1 | Small molecule transport | M14758 | 2.8 |
| c-Kit                       | KIT    | Signaling      | X06182     | 2.7         |
| NADH dehydrogenase (ubiquinone) Fe-S protein 8 | NDUF8S | Metabolism | U65579  | 2.6 |
| cAMP-responsive element modulator | CREM | Signaling      | S68271     | 2.4         |
| Carbonic anhydrase II       | CA2    | Metabolism     | Y00339     | 2.3         |
| Ras-Related protein Rap1b   | RAP1B  | Signaling      | HG3521-HT3715 | 2.1 |
| Phosphoinoside 3-kinase, class 3 | PIK3C3 | Signaling          | Z48973     | 2.1         |
| **Decreased**               |        |                |            |             |
| Mitogen-activated protein kinase-activated protein kinase 3 | MAPKAPK3 | Signaling | U09578 | 2.7 |
| Arginine-rich, mutated in early stage tumors | ARMET | Oncogenesis | M83751 | 2.7 |
| Chromobox homolog 1 (HP1 beta homolog Drosophila) | CBX1 | Chromatin binding | U35451 | 2.3 |

FIG. 3. Specific binding of MEF protein to the IL-8 promoter. EMSAs were performed in A by incubating in vitro translated MEF with a radiolabeled EBS1 probe and an unlabeled EBS1 (lane 2), mEBS1 (lane 3), or EBS2/3 (lane 4) oligonucleotide. In B, MEF (lanes 3, 4, 9, and 10), PEA3 (lanes 5, 6, 11, and 12), or rabbit reticulocyte lysate alone (lanes 1, 2, 7, and 9) were incubated with an EBS1 or EBS2/3 oligonucleotide probe labeled with γ-32P. The reactions were done in the absence or presence of a 100-fold excess of unlabeled competitor oligonucleotide. The specific protein-probe complexes are indicated by arrows. The complexes were separated by electrophoresis on a 6% polyacrylamide gel. The sequence of the probes is indicated below the number of the lanes. In C, cross-linked chromatin was extracted from NB4–306 cells (which constitutively express IL-8) and subjected to immunoprecipitation with the indicated antibodies: input (lane 1), no antibody (lane 2), normal rabbit serum (lane 3), anti-acetyl-histone H3 (lane 4), anti-MEF (lane 5), anti-ELF1 (lane 6), anti-PEA3 (lane 7), anti-PU.1 (lane 8), and anti-ETS2 (lane 9). The length of the PCR-amplified fragment is indicated on the left. Lane M is the size marker. D, cross-linked chromatin was extracted from wild type HEL cells (which lack MEF) and immunoprecipitated with the following antibodies: no antibody (lane 1), normal rabbit serum (lane 2), input (lane 3), anti-acetyl-histone H3 (lane 4), anti-MEF (lane 5), and anti-c-Jun (lane 6).
MEF Regulates IL-8 Expression

Analysis of IL-8 mRNA expression and protein after induction of MEF expression. A, real time PCR was performed with cDNA produced from either HEL/VgRXR or HEL/IND-MEF cells. IL-8 protein production was measured by specific enzyme-linked immunosorbent assays in the culture supernatants of the cells. Cell lines in both assays were incubated with 5 nM ponasterone A for the indicated times. Error bars indicate ±1 S.D.

MEF Expression Increases IL-8 Protein Production—To confirm our microarray data and define the time course of IL-8 expression in response to MEF induction, we performed real time PCR to monitor changes in the level of IL-8 mRNA (Fig. 4A). IL-8 mRNA is expressed at a minimal level in HEL/IND-MEF cells, increases when MEF is induced at 4 h (27.5-fold), and is ~10-fold higher than its baseline at 8 h. Given the profound transactivating effects of MEF on the IL-8 promoter, we sought to determine whether MEF overexpression was sufficient to drive the expression of IL-8 protein in cells. We performed enzyme-linked immunosorbent assays on conditioned media obtained from HEL/IND-MEF cells at various time points after MEF induction (Fig. 4B). IL-8 significantly accumulates in the medium (~52-fold compared with control at 8 h), demonstrating that overexpression of MEF alone is sufficient to induce IL-8 protein expression. The concentration of IL-8 in the medium, which ranged from 1.9 to 6.8 nM during the 24-h period, is biologically relevant as it exceeds the concentration required for high affinity binding to the CXCR1 and CXCR2 IL-8 receptors ($K_d = 2$ nM).

Effect of siMEF on Endogenous IL-8 Expression in NB4–306 Cells—To determine whether MEF is an important regulator of IL-8 expression in vivo, we used RNA interference to knockdown the level of MEF mRNA (and protein) in NB4–306 cells.

For these studies, we used 21-nucleotide siRNA complementary to MEF sequences, whereas siRNA duplexes specific for GFP were used as a control. Introduction of MEF siRNA into NB4–306 cells decreased MEF mRNA levels by ~90% (Fig. 5). This reduction of MEF expression resulted in a 40% decrease in IL-8 mRNA expression, demonstrating the physiologic role of MEF in regulating IL-8 expression.

**DISCUSSION**

The identification of true ETS target genes is complicated by the simplicity of their consensus recognition elements (GGAA), which are present at high frequency throughout genomic DNA and by the great number of related ETS proteins, which are often coexpressed in several different cell types. We have used an inducible expression system in the human HEL leukemia cell line to identify MEF target genes in vivo and have extensively characterized the promoter of one of these genes, namely IL-8. Several of these genes play important roles in hematopoietic cell growth and function including c-kit, phosphatidylinositol-3-kinase, and MDR1, but it will be necessary to further evaluate each of these genes, to examine the importance of MEF in their constitutive or induced expression.

Several of the genes activated by MEF in this system have been reported previously to be regulated by ETS proteins including c-kit (22) and IL-8 (17). We reported previously that MEF can transactivate the IL-3 and GM-CSF promoters in transient transfection studies (1); however, neither gene was identified as an MEF target in this study, possibly because of the lack of specific cofactors required for their induction. In fact, IL-3 and GM-CSF expression was not found in HEL cells by microarray either in the absence or presence of MEF.

We have shown that MEF, and not several other ETS factors, strongly activates IL-8 protein expression. IL-8 is produced by myeloid and lymphoid leukemia cells, both constitutively and in response to 12-O-tetradecanoylphorbol-13-acetate (16, 23). Previous studies have suggested that PEA3 may mediate the induction of IL-8 expression through one of these ETS response elements in hepatoma cells (17). However, no transactivation studies were performed. We have shown that PEA3 binds to IL-8 promoter sequences in vitro, but not in vivo, and it does not activate the promoter. In contrast, overexpression of MEF strongly positively regulates IL-8 expression in hematopoietic cells, and reduction in MEF levels using RNA interference, in hematopoietic cells that express both MEF and IL-8 (NB4–
vivo gene expression and the 3- or 4-nucleotide ETS binding recognition sequence (... GGA(A/T) ... ) present in innumerable locations throughout the genome.

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**Molecular cloning of a third member of the potassium-dependent sodium-calcium exchanger gene family, NCKX3.**

Alexander Kraev, Beate D. Quednau, Stephen Leach, Xiao-Fang Li, Hui Dong, Robert Winkfein, Marco Perizzolo, Xinjiang Cai, RuoMei Yang, Kenneth D. Philipson, and Jonathan Lytton

PAGE 23165:

On line 4, “9q22.1–22.3” should read “9p22.1–22.3.”

**VOLUME 279 (2004) PAGES 6395–6400**

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PAGE 6396:

Under “Experimental Procedures,” subheading “Plasmid Construction”: In the sentence beginning "PEA3 was cloned by reverse transcription . . . ,” the second primer sequence was printed incorrectly. The sentence should read: "PEA3 was cloned by reverse transcription PCR from K562 cells using the Superscript one-step reverse transcription PCR system (Invitrogen) and the following primers: 5'-ATGGAGCG-GAGGATGAAAGC and 5'-AGGGCAACTGGTAGGACAGT."