Transcriptional Regulation of the Human Eosinophil Peroxidase Genes: Characterization of a Peroxidase Promoter

Y.	Yuji	Yamaguchi ^{a,b}
D.G.	Daniel G.	Tenen ^b
S.J.	Steven J.	Ackerman ^b

^aDepartment of Medical Biology and Parasitology, Jichi Medical School, Tochigi-Ken, Japan; ^bDivision of Infectious Disease and Hematology-Oncology, Department of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, Mass., USA

Key Words

IL-3

IL-5

GM-CSF

Abstract

The molecular basis for commitment of progenitors to the eosinophil lineage and mechanisms by which eosinophil-specific genes are expressed and regulated during differentiation is unknown. Expression of eosinophil peroxidase (EPO) is restricted to the eosinophil lineage. To understand the mechanisms involved in transcriptional regulation of EPO gene expression, we cloned the region of the EPO gene upstream of the transcriptional start site and analyzed the cis-acting elements required for EPO promoter activity in an eosinophil-inducible leukemic cell line, HL60-C15. The -1.5 kb EPO-pXP2 promoter construct reproducibly expressed > 120-fold more luciferase activity than did promoterless pXP2, and a 12-fold decrease in promoter activity was obtained when sequences between -122 and -45 bp were deleted. To further characterize regulatory sequences important for promoter activity, we performed linker-scanning analysis on the -122 to -45 bp region and identified a number of positively and negatively acting elements in the promoter.

Correspondence to: Dr. Yuji Yamaguchi, Department of Medical Biology and Parasitology, Jichi Medical School, Minamikawachi-machi, Tochigi, 329-04 (Japan)

Introduction

The eosinophil cationic proteins, major basic protein (MBP), eosinophil cationic proteins (ECP), eosinophil-derived neurotoxin (END), and eosinophil peroxidase (ECP), likely play an important role in the pathogenesis of inflammation and tissue damage in these diseases. The biochemical and functional properties of these cationic proteins have been extensively investigated and their cDNA and genomic DNAs have been cloned and sequenced. Human EPO, a heme-containing glycoprotein, is a member of the peroxidase gene family that induces the closely related horseradish peroxidase, lactoperoxidase, intestinal peroxidase, and neutrophil myeloperoxidase (MPO). The EPO nucleotide sequence is similar to that of other eukaryotic peroxidases and it has been suggested that peroxidases comprised a multigene family that evolved by gene duplication. Like the MPO gene, the human EPO consists of 12 exons and 11 introns spanning about 12 kb of DNA. Expression of the EPO gene at the RNA level has thus far been detected exclusively in the eosinophil lineage and not in other myeloid or non-myeloid hematopoietic lineage. Eosinophil differentiation from precursor cells in the bone marrow is regulated by a number of different cyto-kines, including IL-3, GM-CSF, and IL-5. However, the

© 1994 S. Karger AG, Basel

1018-2438/94/1045-0030

\$5.00/0

molecular basis for the commitment of progenitors to the eosinophil lineage remains unknown. In order to identify transcription factors involved in regulating the commitment and differentiation of progenitors in the eosinophil lineage, we have been analyzing the cis-acting elements and DNA-binding proteins that control gene expression of the eosinophil cell line.

Results and Discussion

Expression of EPO mRNA Is Transcriptionally

Upregulated during n-Butyrate-Induced Eosinophilic

Differentiation of HL-60-C15 Cells

We have previously shown that n-butyrate induces granule formation in HL-60-C15 cells and upregulates the expression of mRNAs encoding the major eosinophil granule-associated proteins, including CLC, ECP, EDN, EPO, and MBP. To determine whether the increased accumulation of EPO mRNA was mediated at the transcriptional and/or posttranscriptional level, we analyzed a message stability experiment, in which actinomycin D was added for the indicated times to both uninduced HL-60-C15 cells and to cells previously induced with n-butyrate for 2 days. The half-life of the EPO mRNA in both uninduced and induced HL-60-C15 cells treated with actinomycin D was 2.5 h, suggesting that the accumulation of EPO mRNA in n-butyrate-induced HL-60-C15 cells was mediated at the transcriptional level. Gruart and co-workers have said that the half-life of EPO mRNA was more than 12 h in umbilical cord blood mononuclear cells induced toward eosinophil differentiation with a combination of hIL-3, hGM-CSF, and hIL-5. In our data, the half-life of EPO mRNA in HL-60-C15 cells was one fifth of that reported by them. One potential explanation for the marked difference in mRNA half-life between the cytokine-induced umbilical cord blood eosinophils and HL-60-C15 cells may be the more rapid cell cycle of the HL-60-C15 leukemic cell line. Alternatively, cytokines such as IL-3, GM-CSF, or IL-5 may enable the developing cord blood eosinophils to stabilize the EPO mRNA. To confirm this finding, we performed nuclear run-on assays in HL-60-C15 cells, both uninduced and induced for 48 h with n-butyrate. Butyrate induced a 2-fold increase in the transcriptional rate of EPO over that of the uninduced cells. These data suggest that expression of the EPO gene is transcriptionally upregulated during butyrate-induced eosinophilic differentiation of the HL-60-C15 subline.

Deletion Analysis of the EPO Promoter Demonstrates the Presence of a Positive Regulatory Element In order to characterize the cis-acting elements required for EPO promoter activity, we have analyzed luciferase (Luc) activity in HL-60-C15 cells transfected with various EPO promoter constructs in the pXP2 expression vector. To localize the positive regulatory elements of the EPO promoter, we produced a series of deletion mutants in the -1.5 kb/EPO-Luc construct using convenient restriction sites and exonuclease III. No significant alteration in promoter activity was observed when sequences between -1.5 kb and -122 bp were deleted. Further deletion to -45 bp produced a greater than 12-fold (90%) decrease in luciferase activity when compared to the -122 bp construct.

The Positively and Negatively Regulatory cis-Elements

Map within the -112/-103 and -62/-43 bp Region of the EPO Promoter Fragment

To identify the proteins binding to the cis-acting elements in the EPO promoter, we designed four 30-bp overlapping oligonucleotides spanning the region between -132 and -43 bp of the promoter and carried out electro-phoretic mobility shift assays. Nuclear extracts from HL-60-C15 cells contained specific proteins which bound to oligomer A (-132 to -102 bp). We performed cross-competition experiments; oligomer A (-132 bp/-102 bp), oligomer B (-112 bp/-82 bp), oligomer C (-92 bp/-62 bp), oligomer D (-72 bp/-42 bp) were added to nuclear extracts as cold competitors prior to the addition of hot oligomer A probe. Only excess oligomer A inhibited the binding of nuclear proteins to the oligomer A probe, whereas the other oligomers were inactive. These results suggest that oligomer A contains a cis-acting element responsible for the majority of EPO promoter activity in the HL-60-C15 eosinophilic subline. To characterize the functional sequence required for EPO promoter activity, we constructed 8 linker scanning mutants by inserting a 10-bp oligonucleotide linker consecutively every 10bp in the promoter. A 3-fold increase in EPO promoter activity was seen when a sequence between -112 and -103 bp was replaced, and a 3-fold decrease in their activity was observed when sequences between -62 bp/-53 bp and -52 bp/-43 bp were mutated.

These experimental data have suggested that the EPO promoter is controlled by negatively and positively regulating cis elements in -122 to -103 bp and -62 to -43 bp of the EPO promoter.