PU.1 Regulates the CXCR1 Promoter*

Nancy C. Wilkinson‡ and Javier Navarro§

From the Department of Physiology and Biophysics and Sealy Center for Molecular Science. University of Texas Medical Branch, Galveston, Texas 77555-0641

(Received for publication, June 8, 1998, and in revised form, October 21, 1998)

The interleukin-8 receptors (CXCR1 and CXCR2) are specifically expressed at high levels in cells of the neutrophil lineage. In this work we identify promoter elements of the CXCR1 gene and the ets family transcription factor PU.1 as a major regulator for activation of the CXCR1 promoter. We first showed that the upstream sequence of CXCR1 (~800 to +86 base pairs (bp)) directs myeloid-specific expression of reporter gene constructs. Second, we showed the presence of negative elements in the sequence from ~800 to ~128 bp and positive elements from ~128 to +50 bp. Third, we demonstrated that the fragment ~22 to +14 bp binds PU.1. Fourth, we showed that PU.1 transactivates the CXCR1 promoter. These data are the first demonstration of PU.1-mediated transcriptional regulation of a neutrophil chemoattractant G protein-coupled receptor.

The molecular mechanisms regulating neutrophil development are not defined yet. Abnormal neutrophil development is related to several pathological conditions such as neutropenia, neutrophilia, and myelogenous leukemia (1–3). The regulation of this developmental process most likely involves the interplay of transcription factors with positive and negative cis-acting elements and cytokines interacting with their cognate receptors (4, 5). For example, disrupting either of the murine genes encoding the transcription factors PU.1 (Sp-1) and CCAAT enhancer-binding protein α impairs neutrophil development (6–8). Similarly, disruption of the gene encoding the cytokine granulocyte colony-stimulating factor (G-CSF)8 inhibits neutrophil development (3). By contrast, disruption of the murine homolog of the chemotactic cytokine interleukin-8 (IL-8) receptor gene (CXCR1) causes neutrophilia (9).

IL-8 receptor subtypes A and B (CXCR1 and CXCR2) are G-protein-coupled receptors expressed at high levels in a myeloid-specific fashion, in mature neutrophils and myeloid precursor cells (10–12). IL-8 suppresses the proliferation of myeloid progenitor and precursor cells via activation of the IL-8 receptor (12, 13). In mature neutrophils, IL-8 is the major mediator for the recruitment of neutrophils from circulation to the sites of injury and infection. Despite the importance of the IL-8 receptor in myeloid development and its restrictive expression in myeloid cells, the transcriptional mechanisms regulating myeloid-specific expression of the IL-8 receptor genes are unknown.

Previous studies have shown that CXCR1 and CXCR2 expression is under transcriptional control (14). The genomic organization and promoter regions of CXCR1 and CXCR2 have been previously mapped (15, 16). The CXCR1 gene consists of two exons interrupted by an intron of ~1.7 kilobases. The entire open reading frame is encoded in exon 2. Sprenger et al. (15) detected promoter activity in the T lymphoma cell line Jurkat when transfected with the chloramphenicol acetyltransferase reporter gene driven by 5’ flanking sequences of the CXCR1 gene (~800 to +21 bp). However, identification of regulatory elements and transcription factors regulating the myeloid lineage-specific expression of the CXCR1 gene have been precluded by the lack of suitable myeloid cell lines expressing high levels of CXCR1 or CXCR2. In this work we delineate cis-acting elements that direct myeloid-specific expression of the CXCR1 promoter and identify the ets family transcription factor PU.1 as a major regulator of the CXCR1 promoter.

EXPERIMENTAL PROCEDURES

Cell Culture—Cell culture supplies were purchased from Life Technologies, Inc. 32Dcl3 cells were a gift from Dr. Joel Greenberger (University of Pittsburgh Medical School, Pittsburgh, PA). NIH3T3, RAW264.7, and CEMT4 cell lines were purchased from the American Type Culture Collection (Rockville, MD). 32Dcl3 cells were cultured in RPMI 1640 medium supplemented with 15% WEHI-3B conditioned media, 15% fetal bovine serum, and 50 IU/ml penicillin-50 µg/ml streptomycin. RAW264.7 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 50 IU/ml penicillin-50 µg/ml streptomycin. CEMT4 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 50 IU/ml penicillin-50 µg/ml streptomycin. NIH3T3 cells were cultured in Dulbecco’s modified Eagle’s medium (1 g/lD-glucose) supplemented with 15% WEHI-3B conditioned media, 10% bovine serum and 50 IU/ml penicillin-50 µg/ml streptomycin. All cell lines were grown at 37 °C and 5% CO2.

Northern Blot Analysis—Total RNA was isolated by the RNAzol procedure (Biotecx Laboratories Inc., Houston, TX). Total RNA (10 µg) was fractionated on 1% agarose-formaldehyde gels and blotted to nylon membranes (Micron Separations Inc., Westborough, MA). The blot was probed with murine PU.1, murine CXCR2, and human β-actin cDNAs labeled by random priming with 50 μCi of [α-32P]dCTP (DuPont NEN). The blot was hybridized at 42 °C for 16–20 h in the following solution: 50% formamide, 5 × SSPE (750 mM NaCl, 500 mM NaH2PO4, 5 mM EDTA, pH 7.4) 5 × Denhardt’s solution, 1% SDS, and 200 µg/ml sonicated calf thymus DNA. Blots were washed three times for 10 min with 0.5 × SSC (75 mM sodium chloride, 7.5 mM sodium citrate) and 1% SDS at 65 °C. The blot for β-actin was washed twice for 10 min with 0.1 × SSC and 1% SDS at 65 °C.

Plasmid Construction—Peripheral blood was collected from healthy human donors and placed in a 1% sodium citrate (anticoagulant) solution. Genomic DNA was extracted from leukocytes as described (17). A forward primer corresponding to ~800 to ~777 bp of CXCR1 and containing an XhoI site (5’-CGGTCGGAGGCCTACAACACCGAAGCTC-3’), and a reverse primer corresponding to ~62 to ~86 bp of CXCR1 and containing a HindIII site (5’-GGACACACCTAAGACCGACCCGGACAGTTGTC-3’) were used to amplify 1.7-kilobase 5’-flanking region of CXCR1 (15). Conditions of PCR included a step of 95 °C for 3 min followed by 25 cycles of 95 °C for 1 min 30 s, 65 °C for 1 min 30 s, and
72 °C for 1 min 30 s, and a final extension of 72 °C for 10 min. The amplified PCR product was gel-purified and digested with XhoI and HindIII, and cloned into SalI-HindIII sites upstream of a luciferase cDNA in a promoterless pGEM-3 vector referred to as pLUC. Deletion constructs were made by digestion with unique restriction sites within the CXCR1 sequence (constructs Ndel, A3 construct, Accl, and A4 construct, PstI). The A2 mutant construct was generated by cassette mutagenesis. The sequence (~58 to +50 bp) was removed at Accl-PstI sites and replaced by a synthetic double-stranded oligonucleotide in which guanine nucleotides at positions -13, -12, and -3 and 4 bp in the wild-type sequence were replaced with thymine nucleotides. The pGEM-3 vector contained the SV40 promoter and enhancer upstream of a luciferase cDNA (pSV40/LUC) was used as a positive control. The β-galactosidase reporter gene driven by the cytomegalovirus promoter (pCMV/β-gal) was used as an internal control to correct for differences in transfection efficiency between experiments. The vectors pSV40/LUC and pCMV/β-gal were provided by Dr. Allan Braiser (University of Texas Medical Branch, Galveston, TX). Flavum was purified by cesium chloride gradient centrifugation and identified from other cell types by centrifugation with monoploy resolving media. Peripheral blood mononuclear cells were collected from healthy human donors and placed in a 1% sodium dodecyl sulfate (SDS) solution. Emitted light was recorded as 2.67 mM MgSO4, 0.1 mM EDTA, 270 mM Tris base, 4.45 mM boric acid, 1 mM EDTA, pH 8.0 as a running buffer. Gels were dried and exposed with intensifying screens to x-ray film for 24–48 h at ~70 °C.

Transfection—Log phase 32Dcl3 (14 × 10^6), RAW264.7 (10^6), CEMT4 (10^6), and NIH3T3 (2.5 × 10^6) cells were incubated with 20 µg of pCMV/LUC or pSV40/LUC constructs and 2 µg of pCMV/β-gal for 5 min at room temperature, electroporated using a 300-V, 960-µF pulse, and then incubated on ice for 15 min. Cells were plated in 5 ml of complete culture media and incubated at 37 °C with 5% CO2. Cells were harvested 17 h after electroporation. Transfected cells were washed three times with phosphate-buffered saline, resuspended in 100 µl of lysis buffer (25 mM Tris-PO4, pH 7.8, 2 mM dithiothreitol, 10% glycerol, 1% Triton X-100), vortexed for 2 min, and stored at -70 °C until ready for reporter gene assays.

Reporter Gene Assays—Luciferase and β-galactosidase assays were performed in duplicate with cell lysates (10 µl) from each transfection. Luciferase assays were performed as described in Promega Protocols and Applications Guide (19). Lysates were incubated with a luciferase assay reagent composed of 20 mM Tricine, 1.07 mM (MgCO3)4MgOH2, 2.67 mM MgSO4, 0.1 mM EDTA, 270 mM Tris base, 4.45 mM boric acid, 1 mM EDTA, pH 8.0 as a running buffer. Gels were dried and exposed with intensifying screens to x-ray film for 24–48 h at ~70 °C.

Transactivation Assays—CEMT4 and NIH3T3 cells were transfected by electroporation with 15 µg of construct A2 (126 to +86 bp), 15 µg of an expression vector encoding for murine PU.1 (CMV/pCB6+PU.1) or vector without the PU.1 (CMV/pCB6+), and 2 µg of pCMV/β-gal. DNA concentrations were adjusted to 42 µg with the CMV/pCB6+ vector. The vector pCMV/pCB6+ PU.1 was generously provided by Dr. Michael Atchison (University of Pennsylvania, Philadelphia, PA).

RESULTS

IL-8 Receptors Are Expressed in Myeloid Precursor Cells—CXCR1 and CXCR2 are highly expressed in neutrophils and readily detected by Northern blot analysis. The short life span of neutrophils and the difficulties of transfecting these cells have precluded the use of neutrophils as a cellular system to elucidate the transcriptional regulation of neutrophil-specific genes. On the other hand, most cell lines of hematopoietic origin express trace amounts of CXCR1 or CXCR2 (23). Recently, we have identified a myeloid cell line, 32Dcl3 (32D), that readily detected the human homolog of CXCR2, two reports have identified the human homolog of CXCR1 in rat and mouse (25, 26). These observations indicate that the murine 32D cell line is a murine interleukin-3-dependent myeloid precursor cell that differentiates into neutrophils in the presence of G-CSF (24). Recently we found that IL-8 and the related chemokine melanoma growth-stimulating activity suppress the proliferation of 32D cells (12). Although previously it was argued that mouse and rat only express the human homolog of CXCR2, two reports have identified the human homolog of CXCR1 in rat and mouse (25, 26). These observations indicate that the murine 32D cell line is a suitable cellular system to identify the regulatory elements and transcription factors regulating the myeloid lineage-specific expression of the human CXCR1 gene.

Myeloid Lineage Specificity of the CXCR1 Promoter—Previous studies showed that CXCR1 promoter constructs containing the first 800 bp of 5′-flanking DNA directed activation of the reporter gene chloramphenicol acetyltransferase in hematopoietic cell lines expressing negligible levels of IL-8 receptors (15, 16). To determine the specificity of the CXCR1 promoter for the myeloid lineage, myeloid (32D and RAW) and nonmyeloid (3T3 and CEM) cell lines were transfected with the -800 to +86 bp/luc promoter construct (A1). As shown in Fig. 2, the A1 promoter construct exhibited the greatest reporter activities in

![Fig. 1. Northern blot analysis of IL-8 receptor mRNA expression.](image)
PU.1 Regulates the CXCR1 Promoter

32D and RAW cell lines, 200- and 400-fold higher than the promoterless luciferase vector, respectively. In the nonmyeloid cells CEM and 3T3, the promoter activity of construct A1 was significantly lower than the activity observed in myeloid cells. Deletion of the sequence -800 to -126 bp (construct A2) increased reporter activity 2-fold relative to construct A1. This finding suggests that the sequence -800 to -126 bp contains negative promoter elements. Further deletion of the sequence -800 to -58 bp (construct A3) still exhibited high levels of promoter activity in the myeloid cells 32D and RAW. Surprisingly, the A3 construct showed higher levels of promoter activity in 3T3 cells than the A1 and A2 constructs, suggesting the presence of negative elements between -126 and -58 bp. Deletion of the -800- to +51-bp region (construct A4) abolished promoter activity. These data indicate the presence of cis-regulating elements between -22 and -14 bp that direct positive promoter activity in the nonhematopoietic cell line 3T3.

The Sequence -22 to +14 bp Binds Nuclear Proteins Present in 32D and RAW Cell Lines—EMSA were performed to identify transcription factors binding to the CXCR1 promoter sequences in a myeloid-specific fashion. EMSAs were performed with 32P end-labeled DNA fragments encompassing the -58- to +50-bp sequence, because the majority of myeloid-specific promoter activity is contained in this region. Only a fragment corresponding to the sequence -22 to +14 bp (A2 probe) produced a fast-migrating, myeloid-specific complex (Fig. 3). An excess of unlabeled A2 probe displaced the binding of the labeled A2 probe to the myeloid-specific factor (Fig. 3, lanes 3 and 7); however, an excess of unlabeled nonspecific probe did not displace the binding of A2 probe (Fig. 3, lanes 11 and 15). A similar myeloid-specific complex was observed with the A2 probe and nuclear extracts from human neutrophils and human monocytes (data not shown). These results suggest that myeloid-specific transcription factors bind to the -22- to +14-bp fragment.

The -22- to +14-bp Fragment Binds to the Hematopoietic Transcription Factor PU.1—Analysis of the -22 to +14 bp sequence revealed consensus binding sequences for transcription factors of the ets family. PU.1 is a member of the ets family that is expressed in myeloid cells, including neutrophils and macrophages, and B cells (27). To determine whether PU.1 binds to the -22- to +14 bp sequence (probe A2), the two putative PU.1 binding sites were first mutated. Disruption of the two putative PU.1 sites (M3) or the PU.1 site proximal to the transcription start site (M2) did not displace the A2 probe bound to the myeloid-specific protein, and M3 and M2 probes did not generate the myeloid-specific complex (Fig. 4). By contrast, disruption of the PU.1 site distal to the transcription start site (M1) effectively displaced the A2 probe bound to the myeloid-specific protein, and the M1 probe generated the myeloid-specific complex (Fig. 4). These findings strongly suggest that the core PU.1 binding motif is located -7 to -4 bp. Second, a fragment corresponding to the PU.1 binding site of the CD11b promoter was an effective competitor for formation of the myeloid-specific complex (Fig. 4, lanes 2-5) but not for nonmyeloid DNA-protein complexes formed with myeloid (Fig. 4, lanes 2-5) and nonmyeloid extracts (Figs. 4, lanes 6-9, and 5A). Third, the PU.1 fragment produced a similar myeloid-specific complex as...
PU.1 Regulates the CXCR1 Promoter

PU.1 Binding Site Is Essential for IL-8 Receptor A Promoter Activity—To determine whether the PU.1 binding site in the -22- to +14-bp sequence is functional, myeloid and nonmyeloid cell lines were transfected with the -226 to +186 bp/luc construct mutated at this site. Disruption of the PU.1 binding site abolished the promoter activity of the -226 to +186 bp/luc construct (A2) (Fig. 7). This result indicates that the PU.1 site is essential for promoter activity and that compensatory elements are not present in this construct to drive the expression of the reporter gene. To directly demonstrate that PU.1 binds and activates the IL-8RA promoter, the nonmyeloid cell lines that do not express PU.1, CEM, and 3T3 were cotransfected with the -126 to +86 bp/luc construct (A2) and an expression vector encoding PU.1. Cotransfections with PU.1 cDNA increased promoter activity 4-fold in CEM cells (Fig. 8A) and >16-fold in 3T3 cells (Fig. 8B) compared with vector alone. These findings strongly suggest that the myeloid-specific expression of the CXCR1 gene is activated by PU.1 interacting with promoter sequences adjacent to the transcription start site.

DISCUSSION

Chemokines are major regulators of the proliferation of myeloid precursor cells (12, 13, 28). However, little is currently known about the mechanisms regulating the expression of chemokine receptors during the commitment and differentiation of progenitor cells toward myeloid lineages and neutrophil development, in particular. The restrictive expression of IL-8 receptors in myeloid precursor cells and neutrophils provides a system to identify the regulatory elements and transcription factors that may regulate the commitment of progenitor cells to the neutrophil lineage.
In this study, the promoter activity of the proximal -800 bp of the CXCR1 gene was analyzed. This sequence was found to contain the regulatory elements that direct CXCR1 promoter activity in a myeloid-specific fashion. High levels of promoter activity were detected specifically in 32D (myeloid precursor cells) and RAW (macrophages). Sequences -800 to -126 bp were found to contain negative regulatory elements. This finding is in agreement with that of Sprenger et al. (15), who suggested the presence of silencer elements between positions -841 and -280 bp on the basis of transfection studies with chloramphenicol acetyltransferase reporter genes in nonmyeloid cell lines. Because most of the promoter activity is localized within the -56- to +50-bp sequence, myeloid-specific proteins binding in this region were identified. On the basis of EMSA, the transcription factor PU.1 was shown to bind the -22- to +14-bp fragment with the common GGAA binding motif at position -7 to -4 bp. Disruption of the PU.1 binding site abolished the myeloid-specific transcriptional activity of the CXCR1 promoter. Transfection of nonmyeloid cell lines CEM (T cells) and 3T3 (fibroblasts) with CDNA encoding PU.1 increased the promoter activity of the -126 to +86 bp/luc construct. Because PU.1 expression in nonmyeloid cell lines produced high levels of promoter activity, this suggests that PU.1 does not require other myeloid-specific factors for activation of the promoter construct. These data show for the first time the transcriptional regulation of a chemotactic G protein-coupled receptor by the myeloid transcription factor PU.1.

PU.1 has been shown to regulate several myeloid lineage-specific genes, including granulocyte-macrophage CSF receptor, G-CSF receptor, macrophage CSF receptor, CD11b, scavenger receptor, FeRIIIa, FeR1b, c-fes, interleukin-1β, myeloperoxidase, and neutrophil elastase (8, 29–32). Furthermore, PU.1 has also been found to control neutrophil development. Studies with PU.1-null mice indicate that PU.1 is necessary for normal neutrophil development (6–7, 33–35). Neutrophils from mice deficient of PU.1 fail to terminally differentiate and are functionally incompetent. PU.1-/- mice contain neutrophils that do not respond to IL-8, indicating that functional receptors are not expressed (33) and further supporting the view that PU.1 is required for the expression of IL-8 receptors.

Our studies reveal that the regulatory sequences analyzed in this study do not direct cell-specific expression, because high levels of promoter activity are demonstrated in both 32D cells, which express IL-8 receptors, and RAW cells, which do not express IL-8 receptors. Similar findings were observed with the promoter of the eosinophil-specific IL-5 receptor, in which high levels of promoter activity were obtained in both myeloid and eosinophilic cell lines (36). The neutrophil lineage-specific expression of CXCR1 transcripts could possibly be attributable to post-transcriptional mechanisms or transcription factor regulatory sites located further upstream or downstream of the sequences analyzed in this study. Further experiments will be focused on mapping additional functional elements of the CXCR1 promoter and identifying the transcription factors that bind to these elements to elucidate the mechanisms regulating the neutrophil lineage-specific expression of CXCR1.

Acknowledgments—We thank Dr. Shyam Ramakrishnan for assistance with the promoter constructs, Dr. Allan Braiser for luciferase and β-galactosidase reporter gene vectors, and Dr. Micheal Atchison for the PU.1 expression vector.

REFERENCES
1. Holyoake, T. L., Freshney, M. G., Sproul, A. M., Richmond, L. J., Alcorn, M. J., Steward, W. P., Fitzsimons, E. E., Dunlop, J. D., Franklin, I. M., and Pragnell, I. B. (1993) Stem Cells 11, Suppl. 3, 122–129.
2. Dong, F., Hoefsloot, L. H., Schelen, A. M., Breders, L. C., Meijer, Y., Veerman, A. P. Tuw, I. P., and Lowenberg, B. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 4480–4489.
3. Lieszke, G. J., Grail, D., Hodgson, G., Mitchell, D., Stanely, E., Cheers, C., Fowler, K. J., Basu, S., Zhang, Y. F., and Dunn, A. R. (1994) Blood 84, 1737–1743.
4. Lenny, N., Westendorf, J. J., and Hiebert, S. W. (1997) Mol. Biol. Rep. 24, 157–168.
5. Lieszke, G. J. (1997) Ciba. Found. Symp. 204, 60–74.
6. Scott, E. W., Simon, C., Anastasi, J., and Singh, H. (1994) Science 265, 1373–1377.
7. McKechnie, S. R., Torbett, B. E., Anderson, K. L., Henkel, G. W., Vestal, D. J., Baribault, H., Klemas, M., Feeney, A. J., Wu, G. E., Paige, C. J., and Maki, R. A. (1996) EMBO J. 15, 5647–5658.
8. Zhan, Z. L., Zhang, P. X., Wang, X. D., Hetherington, C. J., Darlington, G. J., and Tenen, D. G. (1996) Proc. Natl. Acad. Sci. U. S. A. 94, 569–574.
9. Casalecco, G., Lee, J., Kikky, K., Ryan, A. M., Pitts-Meek, S., Hultgren, B., Wood, W. I., and Moore, M. W. (1994) Science 265, 682–684.
10. Thomas, K. M., Taylor, L., and Navarro, J. (1991) J. Biol. Chem. 266, 14839–14841.
11. Prado, G. P., Thomas, K. M., Suzuki, H., LaRosa, G. J., Wilkinson, N., Foles, E., and Navarro, J. (1994) J. Biol. Chem. 269, 12391–12394.
12. Sanchez, X., Hodges-Cousins, B., Horton, J., and Navarro, J. (1998) J. Immunol. 160, 906–910.
13. Brumley, H. E., Cooper, S., Casalecco, G., Hage, N. L., Baille, E., and Moore, M. W. (1996) J. Exp. Med. 182, 1845–1852.
14. Lloyd, A. R., Biragyn, A., Johnston, J. A., Taub, D. B., Xu, L., Michiel, D., Sprenger, H., Oppenheim, J. J., and Kelvin, D. J. (1995) J. Biol. Chem. 270, 28145–28192.
15. Sprenger, H., Lloyd, A. R., Meyer, R. G., Johnston, J. A., and Kelvin, D. J. (1994) J. Immunol. 153, 2524–2532.
16. Abuja, S. K., Shetty, A., Tiffany, H. L., and Murphy, P. M. (1994) J. Biol. Chem. 269, 11065–11072.
17. John, S. W. M., Weitzner, G., Rozen, R., and Scriver, C. R. (1991) Nucleic Acids Res. 19, 408.
18. Ausabul, F. M., Brent, R., Kingston, R. E., Moore, D. D., Sheldman, J. G., Smith, J. A., and Strul, K. (eds) (1987) Current Protocols in Molecular Biology, Vol. 2, pp. 6.66–6.67, John Wiley & Sons, New York.
19. Titus, D. (ed) (1991) Promega Protocols and Applications Guide, 2nd Ed., pp. 101–102, Promega, Madison, WI.
20. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, pp. 16.66–16.67, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
21. Johnson, D. R., Lennard, S., and Bale, A. E. (1995) Anal. Biochem. 232, 248–254.
PU.1 Regulates the CXCR1 Promoter

23. Moser, B., Barella, L., Mattei, S., Schumacher, C., Boulay, F., Colombo, M. P., and Baggioiini, M. (1993) *Biochem. J.* **294**, 285–292
24. Valtieri, M., Tweardy, D. J., Caracciolo, D., Johnson, K., Mavilio, F., Altmann, S., Santoli, D., and Rovera, G. (1987) *J. Immunol.* **138**, 3829–3835
25. Dunstan, C. A. N., Salafranca, M. N., Adhikari, S., Xia, Y., Feng, L., and Harrison, J. K. (1996) *J. Biol. Chem.* **271**, 32770–32776
26. Bozic, C. R., Gerard, N. P., von Uexkull-Goldenband, C., Kolakowski, Jr., L. F., Conklyn, M. J., Breslow, R., Showell, H. J., and Gerard, C. (1994) *J. Biol. Chem.* **269**, 29355–29358
27. Chen, H.-M, Zhang, P., Voso, M. T., Hohaus, S., Gonzalez, D. A., Glass, C. K., Zhang, D.-E., and Tenen, D. G. (1996) *Blood.* **85**, 2918–2928
28. Rollins, B. J. (1997) *Blood.* **90**, 909–928
29. Ford, A. M., Bennett, C. A., Healy, L. E., Towatari, M., and Greaves, M. F. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 10838–10843
30. Oelgeschlager, M., Nuchprayoon, I., Luscher, B., and Friedman, A. D. (1996) *Mol Cell. Biol.* **16**, 4717–4725
31. Smith, L. T., Hohaus, S., Gonzalez, D. A., Dziennis, S. E., and Tenen, D. G. (1996) *Blood.* **88**, 1234–1247
32. Heydemann, A., Boehmler, J. H., and Simon, M. C. (1997) *J. Biol. Chem.* **272**, 29527–29537
33. Anderson, K. L., Smith, K. A., Pio, F., Torbett, B. E., and Maki, R. A. (1998) *Blood.* **92**, 1576–1585
34. Anderson, K. L., Smith, K. A., Connors, K., McKercher, S. R., Maki, R. A., and Torbett, B. E. (1998) *Blood.* **91**, 3702–3710
35. DeKoter, R. P., Walsh, J. C., and Singh, H. (1998) *EMBO J.* **17**, 4456–4468
36. Sun, Z., Yergeau, D. A., Tuyvens, T., Tavernier, J., Paul, C. C., Baumann, M. A., Tenen, D. G., and Ackerman, S. J. (1995) *J. Biol. Chem.* **270**, 1462–1471