1.2 m*M* $MgSO_4$, 15 m*M* sodium acetate, 10 m*M* glucose, and bovine serum albumin, 10 mg/ml.

21. The medium added to vary the *p*H was 120 m*M* NaCl, 4.5 m*M* KCl, 1 m*M* EDTA, 1.2 m*M* $MgSO_4$, 10 m*M* glucose, bovine serum albumin, 10 mg/ml; buffered with 100 m*M* *N*-(2-hydroxyethyl)piperazine-*N*′-3-propanesulfonic acid, *p*H 7.8, or 100 m*M* *N*-(2-acetamido)-2-aminoethanesulfonic acid, *p*H 6.8, or 100 m*M* 1-(*N*-morpholino)ethanesulfonic acid, *p*H 6.0.

22. We are especially grateful to A. Ullrich (Genentech Corporation) for supplying the insulin receptor cDNA probe. Without his collaboration, we would not have been able to do these studies. We also acknowledge the cooperation of M. J. Elders with whom we have collaborated in previous studies of this patient. In addition, we thank many colleagues at NIH for helpful advice and discussions: D. Accili, C. Frapier, P. Gorden, M. Kasuga, S. Lewis, V. Moncada, M. Rechler, C. T. Roberts, Jr., J. Roth, and M. Zasloff. D. Hamer generously supplied the bovine papilloma expression vector used for the transfection experiments. The oligonucleotides used in these studies were synthesized by P. Fast, J. Jensen, J. Regan, and K. B. Seamon. C.L.B. was recipient of a Pharmacology Research Associate Training Fellowship from the National Institute of General Medical Sciences. Supported in part by Juvenile Diabetes Foundation International (Mortimer B. Lipsett Memorial Research Grant).

2 February 1988; accepted 18 March 1988

Translocation and Rearrangement of Myeloperoxidase Gene in Acute Promyelocytic Leukemia

SUSAN C. WEIL,* GAYLE L. ROSNER, MARTHA S. REID, REX L. CHISHOLM, RICHARD S. LEMONS, MAURICE S. SWANSON, JOHN J. CARRINO, MANUEL O. DIAZ, MICHELLE M. LE BEAU

Acute promyelocytic leukemia (subtype M3) is characterized by malignant promyelocytes exhibiting an abundance of abnormally large or aberrant primary granules. Myeloperoxidase (MPO) activity of these azurophilic granules, as assessed by cytochemical staining, is unusually intense. In addition, M3 is universally associated with a chromosomal translocation, t(15;17)(q22;q11.2). In this report, the *MPO* gene was localized to human chromosome 17 (q12–q21), the region of the breakpoint on chromosome 17 in the t(15;17), by somatic cell hybrid analysis and in situ chromosomal hybridization. By means of *MPO* complementary DNA clones for in situ hybridization and Southern blot analysis, the effect of this specific translocation on the *MPO* gene was examined. In all cases of M3 examined, *MPO* is translocated to chromosome 15. Genomic blot analyses indicate rearrangement of *MPO* in leukemia cells of two of four cases examined. These findings suggest that *MPO* may be pivotal in the pathogenesis of acute promyelocytic leukemia.

MYELOPEROXIDASE (MPO) IS A critical bacteriocidal protein with molecular mass of approximately 150 kD (*1*). MPO is the most abundant protein of the mature human polymorphonuclear neutrophil, accounting for 3 to 5% of the dry weight of the cell (*2*). We (*3*) and others (*4, 5*) have demonstrated that *MPO* is abundantly expressed only at the promyelocyte stage; the amount of *MPO* rapidly declines as myeloid cells proceed toward terminal differentiation. The appearance of MPO in early granulocytes (*6–8*) serves as a lineage-specific marker of both normal and malignant myeloid differentiation. As a clinical diagnostic tool, MPO cytochemical activity is critical in distinguishing poorly differentiated malignant blast cells of acute myelogenous leukemia (AML), which have MPO activity, from the blast cells in acute lymphoblastic leukemia (ALL), which do not have MPO activity (*9*), and in assigning acute leukemia cases to one of several subtypes according to the French-American-British (FAB) system.

Acute promyelocytic leukemia (APL) (FAB classification, M3) is a distinct, well-characterized, clinical, morphologic, and cytogenetic subtype of AML. Morphologically, intense granulation with abnormally large or bizarre primary granules, kidney-shaped nuclear morphology, and abundant Auer bodies are characteristic of the malignant promyelocytes associated with this disorder. Intensely positive MPO activity in APL, as assessed by cytochemical staining, suggests an abundance of functional MPO. Cytogenetic analysis consistently reveals a translocation involving chromosomes 15 and 17 in M3 cells. This specific translocation, t(15;17)(q22;q11.2), has not been observed in any other subtype of AML or in any other malignant disease (*10*). The apparently high levels of MPO seen in APL raise the possibility that *MPO* may be affected by this translocation and, therefore, may be involved in the pathogenesis of this malignancy. We describe herein the use of *MPO* complementary (cDNA) clones as hybridization probes to localize *MPO* to normal metaphase chromosomes, to examine its location in metaphase cells from M3 leukemia, and to analyze its genomic organization by Southern blotting of DNA from normal and M3 cells.

A cDNA clone (pHMP7) was isolated by immunologic screening with rabbit antiserum raised against purified MPO and characterized as an authentic *MPO* cDNA clone as described (*3*). This clone was subsequently used to isolate two longer cDNA clones by nucleic acid hybridization to the original cDNA libraries; these were subcloned into the plasmid vector pGEM-3 blue (Promega Biotech). Comparison of the restriction endonuclease maps of these clones (Fig. 1) with published data (*11*) indicates that our clones pHMP10A and pHMP2E cover the 3′ end of *MPO*. Our clones represent both

Table 1. Synteny test of the *MPO* gene and human chromosomes in rodent × human hybrid clones. Somatic cell hybrids were scored for the presence (+) or absence (−) of specific human chromosomes by gene-enzyme and cytogenetic analysis and the presence or absence of human *MPO* coding sequences by hybridization or radiolabeled *MPO* cDNA to Southern blots as described (*12*).

Human chromosome	*MPO* gene/chromosome				% Asynteny
	+/+	+/−	−/+	−/−	
1	16	14	3	1	50
2	9	18	3	0	70
3	1	9	0	1	82
4	7	3	1	0	57
5	15	11	1	2	41
6	17	10	3	1	42
7	0	9	0	1	90
8	15	12	3	0	50
9	7	2	4	0	46
10	6	6	0	0	50
11	21	3	5	0	28
12	15	9	3	1	43
13	21	5	2	1	24
14	20	7	3	0	33
15	19	13	2	2	42
16	11	0	8	1	40
17	28	0	0	19	0
18	1	11	0	0	92
19	16	11	1	3	39
20	10	8	0	4	36
21	0	11	0	0	100
22	0	12	0	1	92
X	10	12	1	2	52

S. C. Weil, G. L. Rosner, M. S. Reid, R. L. Chisholm, Departments of Pathology, Medicine, and Cell Biology, Northwestern University Medical Center, Chicago, IL 60611.
R. S. Lemons, Department of Human Genetics, University of Utah Medical Center, Salt Lake City, UT 84132.
M. S. Swanson, Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60201.
J. J. Carrino, M. O. Diaz, M. M. Le Beau, Department of Medicine, University of Chicago, Chicago, IL 60637.

*To whom correspondence should be addressed.

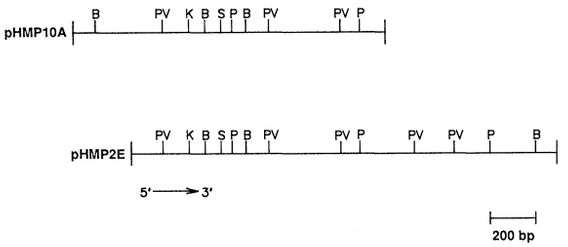

Fig. 1. Restriction maps of *MPO* cDNA clones pHMP10A and pHMP2E. Restriction endonuclease sites are as indicated: Bgl II (B), Kpn I (K), Pst I (P), Pvu II (PV), and Sma I (S). Orientation (5′ → 3′) was determined by comparison of restriction maps to sequences and maps of *MPO* clones isolated by others independently (*11*).

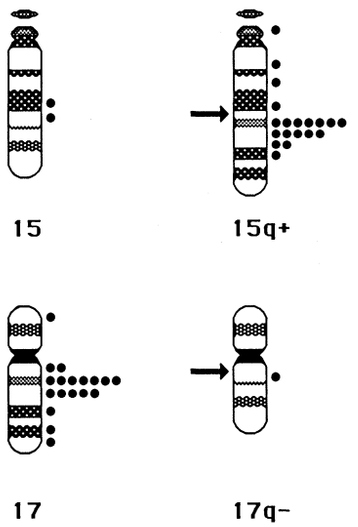

Fig. 2. Distribution of labeled sites on normal homologs 15 and 17 and on the translocation derivatives 15q+ and 17q−, after hybridization of a *MPO*-specific probe (pHMP10A) to metaphase cells from a bone marrow aspirate of an APL patient (Table 2, patient 1) who had a t(15;17). One hundred metaphase cells from this hybridization were examined. The arrows identify the breakpoint junctions on the rearranged homologs. The result of this hybridization indicated that *MPO* is translocated to the rearranged chromosome 15 and, therefore, is distal to the breakpoint of the t(15;17).

3′ coding and noncoding sequences of *MPO* cDNA. Clone pHMP2E extends farther in the 3′ direction. Additional confirmation of the identity of these clones as true myeloperoxidase cDNA was obtained by Northern blot analysis, in which each of these clones hybridized to two HL-60 cell polyadenylated RNA species of approximately 3.6 and 2.9 kb (*3*), the reported sizes for *MPO* mRNA transcripts.

For chromosomal localization of *MPO*, we used the pHMP10A and pHMP2E clones for the analysis of a panel of rodent × human somatic cell hybrids (*12*) and for in situ chromosomal hybridizations. Southern blot analysis of the hybrid panel showed concordance between the presence of human *MPO* sequences and human chromosome 17 (Table 1). Of the primary hybrid clones back-selected from HAT medium into bromodeoxyuridine (BrdU)-containing medium, all lost human chromosome 17 by segregation and concordantly lost *MPO* DNA sequences. In situ hybridization of the *MPO* pHMP2E probe to normal human metaphase chromosomes derived from peripheral blood lymphocytes resulted in specific labeling only of chromosome 17 (*13*). The labeled sites on this chromosome were clustered at 17q11–q22.

To determine the location of *MPO* relative to the breakpoint on chromosome 17 in the t(15;17), we hybridized the *MPO* probes to metaphase cells obtained from bone marrow aspirates of five APL patients (Table 2 and Fig. 2). In this analysis, we observed labeling on the normal chromosome 17 at bands q11–q21 and on the 15q+ chromosome at bands q12–q21 of the material translocated from chromosome 17. The normal chromosome 15 homolog and the 17q− chromosome were not specifically labeled. Thus, *MPO* (or the portions detectable by our probes) is translocated from chromosome 17 to chromosome 15 in M3 cells. The breakpoint of the t(15;17) appears to be in 17q11.2; thus, these results allowed us to narrow the localization of *MPO* to 17q12–q21.

To detect rearrangements of *MPO* at the molecular level, we prepared Southern blots from high molecular weight DNA digested by Bam HI, Bgl II, Eco RI, or Hind III. The DNA was prepared from bone marrow cells from four M3 patients with a t(15;17), from HL-60 cell DNA, and from normal human peripheral blood cell DNA. These blots were hybridized with ^{32}P-labeled Eco RI–digested insert from clone pHMP10A. Southern blot analysis (Fig. 3) showed hybridization of the *MPO* probe to bands of 9.8 kb in Bam HI digests, 7.6 and 2.5 kb in Bgl II digests, 19 kb in Hind III digests, and >24 kb in the Eco RI digests of normal peripheral blood or HL-60 cell DNA. Although HL-60 cells represent a pure population of malignant promyelocytes, they do not exhibit the t(15;17) seen in APL. Two new bands not present in normal DNA from two individuals or in HL-60 cell DNA appeared in the Bam HI and Bgl II digests of DNA from leukemia cells of patient L1. The respective sizes of these bands were 3.3 and 3.6 kb. These novel bands were not present in DNA prepared from bone marrow cells of the same patient obtained during a complete remission, thus ruling out restriction fragment length polymorphism (RFLP) of *MPO* as the source of these novel bands. In order to rule out plasmid contamination, the blot was stripped and reprobed with ^{32}P-labeled nonrecombinant pGEM-3 blue plasmid. The bands noted previously with the *MPO* cDNA insert were not detected.

Table 2. In situ hybridization of *MPO* probes to leukemia metaphase cells from five patients with a t(15;17).

Patient no.	Total number of cells analyzed	Number of labeled sites	Number of labeled sites (%)						
			Normal 15	15q+	15q+ breakpoint junction*	Normal 17	Bands 17q11–q22†	17q−	17q− breakpoint junction*
					pHMP2E probe				
1	100	112	3 (2.7%)	12 (10.7%)‡	11 (9.8%)	12 (10.7%)‡	11 (9.8%)	1 (0.9%)	1 (0.9%)
					pHMP10A probe				
1	100	152	2 (1.3%)	19 (12.5%)‡	16 (10.5%)	18 (11.8%)‡	15 (9.9%)	1 (0.7%)	1 (0.7%)
2	100	180	8 (4.4%)	20 (11.1%)‡	15 (8.3%)	18 (10%)‡	15 (8.3%)	6 (3.3%)	3 (1.7%)
3	100	118	3 (2.5%)	11 (9.3%)‡	10 (8.5%)	13 (11.0%)‡	12 (10.2%)	3 (2.5%)	1 (0.9%)
4	55	83	1 (1.2%)	8 (9.6%)‡	8 (9.6%)	12 (14.5%)‡	10 (12.1%)	4 (4.8%)	0
5	100	120	1 (0.8%)	10 (8.3%)‡	8 (6.7%)	13 (10.8%)‡	11 (9.2%)	2 (1.7%)	0

*The breakpoint junction of the 15q+ chromosome consists of bands 15q21–q22 and 17q12–q21; the breakpoint junction of the 17q− chromosome consists of bands 17q11 and 15q22–q24. †Refers to bands q11–q22 of the normal 17 homolog. ‡Significantly different from expectation (Poisson distribution) $P < 0.0005$. The mean was estimated from the number of labeled sites on all chromosomes except the normal 17 and 15q+ chromosomes.

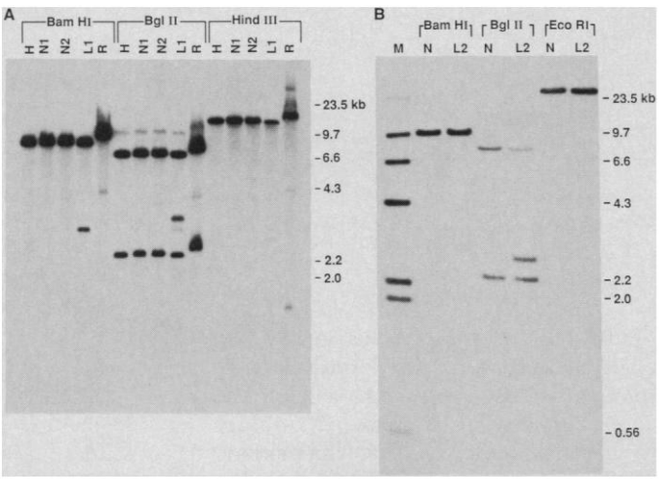

Fig. 3. Southern blots of high molecular weight DNA (*16*) isolated from bone marrow cells of four M3 patients with a t(15;17), HL-60 cells (H), or normal human peripheral blood cells (N). Results from the first patient with leukemia (L1) and in remission (R) are shown in (**A**). Case L2 is shown in (**B**). After restriction endonuclease digestion with Bam HI, Bgl II, Eco RI, or Hind III, the DNA fragments were separated, transferred, and hybridized as described to *MPO* probe pHMP10A (*17*). The sizes of the marker bands are given in kilobases. The ^{32}P-labeled probes were prepared by random primer (*18*) labeling of pHMP10A cDNA insert to a specific activity of approximately 1×10^9 dpm per microgram.

In patient L2, only Bgl II–digested DNA had a novel band of molecular weight 3.1 kb as compared to DNA from normal cells. Plasmid contamination was ruled out because in this experiment the probe did not detect 20 pg of plasmid DNA that had been separated by electrophoresis and blotted. Although no remission DNA from this patient was available, polymorphism at the *MPO* locus appears to be a rare phenomenon, since restriction enzyme mapping with Bgl II and DNA from 12 hematologically normal, unrelated individuals revealed no RFLPs when the *MPO* cDNA insert was used as a probe (*14*). Two additional individuals with APL (L3 and L4) did not show novel bands when their DNA was analyzed by Southern blot analysis.

One explanation for the presence of these novel bands in two of the M3 patients examined is that the breakpoint on chromosome 17 of the t(15;17) occurs within the *MPO* sequences. Alternatively, M3-associated rearrangements of the *MPO* gene such as submicroscopic deletions or inversions that are unrelated to the translocation breakpoint may have occurred. The lack of novel bands in the other two patients does not exclude the possibility of rearrangements either related or unrelated to the chromosomal breakpoint occurring outside the *MPO* sequences encompassed by the pHMP10A probe, as our cDNA probes are not full length. Break sites can also occur in genomic sequences not encompassed by cDNA probes.

There is a good deal of evidence demonstrating the remarkable specificity of certain chromosomal rearrangements with particular morphologic subtypes of leukemia and lymphoma. The t(15;17) has not been observed in any subtypes of AML other than M3. The analysis of variant translocations in M3 that involve three chromosomes suggests that the 15q+ chromosome is the critical recombinant chromosome (*15*). Our results indicate that most or all of *MPO* on chromosome 17 is translocated to chromosome 15 and, thus, is present on the critical rearranged chromosome. If *MPO* is rearranged as a result of the t(15;17), this actively expressed gene may be involved in the activation of an as yet unknown proto-oncogene on chromosome 15. In conclusion, translocation and rearrangement of *MPO* may be implicated in the pathogenesis of a subtype of acute granulocytic leukemia.

REFERENCES AND NOTES

1. M. Yamada, M. Mari, T. Sugimura, *Biochemistry* **20**, 766 (1981).
2. S. J. Klebanoff, *Ann. Int. Med.* **93**, 480 (1980).
3. S. C. Weil *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2057 (1987).
4. H. P. Koeffler, J. Raynard, M. Dertcheck, *Blood* **65**, 484 (1985).
5. M. Yamada and K. Kurahashi, *J. Biol. Chem.* **259**, 3021 (1984).
6. D. F. Bainton and M. G. Farquhar, *J. Cell Biol.* **28**, 277 (1966).
7. K. B. Pryzwansky, L. E. Martin, J. K. Spitznagel, *J. Reticuloendothelial. Soc.* **24**, 295 (1978).
8. E. Cramer, K. B. Pryzwansky, J. Villeval, V. Testa, J. Breton-Gorius, *Blood* **65**, 423 (1975).
9. J. M. Bennett *et al.*, *Br. J. Haematol.* **33**, 451 (1976).
10. J. D. Rowley *et al.*, *Int. J. Cancer* **20**, 869 (1977); R. A. Larson *et al.*, *Am. J. Med.* **76**, 827 (1984); Fourth International Workshop on Chromosomes in Leukemia, 1982, *Cancer Genet. Cytogenet.* **11**, 288 (1984).
11. K. R. Johnson *et al.*, *Nucleic Acids Res.* **15**, 1023 (1987).
12. Somatic cell hybrids were formed by polyethylene glycol–mediated fusion of human VA2, A549, and IMR90 fibroblast cells to Syrian BHK-B1 hamster cells that were mutant in their thymidine kinase gene, permitting hybrid selection with HAT medium (hypoxanthine, aminopterin, and thymidine). Because human cells cannot survive in 10 μM ouabain, hybrids were initially grown in HAT-ouabain medium. BHK-B1 hamster × human hybrids selected in HAT medium retain human chromosome 17 as a result of the mutant hamster thymidine kinase gene. To derive cells that have lost human chromosome 17 by segregation, we removed some primary hybrid clones from HAT medium; these were grown in HT medium lacking aminopterin, and then in medium containing 10 μM BrdU. Cells that survive in BrdU-containing medium have lost human chromosome 17 and the thymidine kinase gene by segregation. A panel for mapping studies was established from a series of hybrids that contain the entire rodent genome but have selectively lost various combinations of human chromosomes. The human chromosome content of these hybrid lines was determined by screening for up to 34 gene enzyme systems and, in selected cases, by cytogenetic analyses with trypsin-Giemsa banding. Hybrid clones back-selected into BrdU-containing medium were confirmed by gene enzyme analysis of human galactokinase to have lost human chromosome 17 as a result of segregation. High molecular weight DNA for Southern blot analysis and cell homogenates for isozyme studies were prepared from the same passages of cells. The MPO clone was radiolabeled with ^{32}P by use of random oligonucleotide primers and the Klenow fragment of DNA polymerase I, to a specific activity of 1×10^8 to 4×10^8 dpm per microgram of DNA and annealed to Southern blots of Hind III– or Eco RI–digested DNAs from the hybrid cell panel. S. J. O'Brien, J. M. Simonson, E. Eichelberger, in *Techniques in Somatic Cell Genetics*, J. W. Shay, Ed. (Plenum, New York, 1982), pp. 513–524; R. S. Lemons, W. G. Nash, S. J. O'Brien, R. E. Benveniste, C. J. Sheer, *Cell* **14**, 995 (1978).
13. M. M. Le Beau, C. A. Westbrook, M. O. Diaz, J. D. Rowley, *Nature (London)* **312**, 70 (1984). Radiolabeled pHMP2E cDNA probe was prepared by nick-translation of the entire plasmid with all four ^{3}H-labeled deoxynucleoside triphosphates to a specific activity of 1.0×10^8 to 2.0×10^8 dpm per microgram. Metaphase cells were hybridized at 4.0 and 8.0 ng of probe per milliliter of hybridization mixture. Autoradiographs were exposed for 11 days. Of 100 metaphase cells examined, 25 (25%) were labeled on region q1 or q2 of one or both chromosomes 17. Of 137 labeled sites observed, 28 (20.4%) were located on this chromosome. These sites were clustered at bands q11 to q22, and this cluster represented 75% of the grains of chromosomes 17 and 15.3% (21/137) of all labeled sites [significantly different from expectation (Poisson distribution), $P < 0.0005$]. The largest number of grains were located at 17q12–q21. Similar results were obtained in hybridizations in which we used the other *MPO*-specific probe, pHMP10A. All hybridizations were repeated twice and gave similar results in each experiment.
14. T. Miki, S. C. Weil, G. L. Rosner, M. S. Reid, K. K. Kidd, *Nucleic Acids Res.*, in press.
15. J. D. Rowley, *Cancer Res.* **44**, 3159 (1984); S. Misawa, E. Lee, C. A. Schiffer, Z. Liu, J. R. Testa, *Blood* **67**, 270 (1986); M. M. Le Beau *et al.*, *Leukemia* **1**, 795 (1987).
16. N. Blin and D. W. Stafford, *Nucleic Acids Res.* **3**, 3203 (1976).
17. E. A. Shima *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 3439 (1986).
18. A. Feinberg and B. Vogelstein, *Anal. Biochem.* **137**, 226 (1984).

19. We express our gratitude to J. D. Rowley whose initial recognition of the t(15;17) along with encouragement, support, and helpful discussions were essential to our work. We thank D. G. Scarpelli for continued encouragement and support, P. Adams for secretarial assistance, and J. K. Spitznagel for contributions toward the cloning of *MPO*. The technical assistance of R. Espinosa III is gratefully acknowledged. S.C.W. was a recipient of NIH grant 1R01 CA44475 and American Cancer Society, Illinois Division, research grant 86–50 during the course of this work. Supported also by Department of Energy contract DE-FG02-86ER60408 and USPHS grant CA16910 (to J. D. Rowley), University of Chicago Cancer Research Foundation (M.O.D. and M.M.L.), and NIH grant CA09273 Environmental Biology Training Grant (J.J.C.). M.M.L. is a Scholar of the Leukemia Society of America.

12 November 1987; accepted 26 February 1988.