Molecular Cloning, Expression, and Chromosomal Localization of a Human Gene Encoding the CD33 Myeloid Differentiation Antigen

By Stephen C. Peiper, Richard A. Ashmun, and A. Thomas Look

Monoclonal antibodies of the CD33 cluster group recognize a 67-kilodalton (Kd) protein, designated p67, expressed on the surface of normal human myeloid progenitors and leukemic cells from most patients with acute myelogenous leukemia. The human gene encoding p67 was isolated in a mouse genetic background after DNA-mediated gene transfer and fluorescence-activated cell sorting (FACS) for transformants that bound the monoclonal antibody MY9. After three serial rounds of gene transfer and cell sorting, multiple independently derived tertiary mouse cell transformants were obtained that expressed p67. Southern blot analysis revealed that these transformants shared restriction fragments containing highly reiterated human DNA sequences. Two shared EcoRI fragments of 3.3-kilobase (kb) and 9.5-kb pairs were molecularly cloned into bacteriophage vectors. A subsegment of the 3.3-kb fragment lacking repeated sequences was then used as a unique sequence probe to isolate two independent cosmids clones.

GLYCOPROTEINS are expressed on the plasma membrane of hematopoietic cells in patterns that reflect their state of differentiation and maturation. Monoclonal antibodies have been developed that bind to these “differentiation antigens” on the surface of restricted subsets of human myeloid cells. Approximately 14 different cluster group specificities of monoclonal antibodies that bind surface molecules of myeloid cells have been identified as part of a recent international workshop. In general, antibodies from these cluster groups cross react with human cells outside the myeloid lineage. Only antibodies of the CD33 cluster group (MY9, L1B2, and L4F3) recognize an antigen expressed exclusively by myeloid cells. The 67-kilodalton (Kd) protein immunoprecipitated by these antibodies, designated p67, is expressed on colony-forming units for granulocytes, erythrocytes, monocytes, and megakaryocytes (CFU-GEMM), progenitors of granulocytes and mononuclear phagocytes (CFU-GM), early erythroid progenitors (BFU-E), mast cells, and leukemic myeloblasts from ~85% of patients with acute myelogenous leukemia (AML). In contrast, p67 is not expressed by terminally differentiated granulocytes, normal or malignant lymphoid cells, or nonhematopoietic cells. Thus, CD33 monoclonal antibodies are helpful diagnostic reagents in cases of undifferentiated AML that cannot be distinguished by cytochemical staining from acute lymphoblastic leukemia (ALL). The MY9 and L4F3 monoclonal antibodies have also been used therapeutically to purge minimal residual leukemic myeloblasts from autologous bone marrow grafts given to patients with AML in remission, since the protein recognized by these antibodies is expressed by most leukemic myeloblasts but not by primitive normal hematopoietic stem cells.

Despite interest in CD33 antibodies as diagnostic and therapeutic reagents, little is known about the biochemical features or biologic functions of p67. We undertook molecular cloning of the gene encoding p67 to gain insight into the function of this membrane protein and the mechanisms that regulate its expression. The p67 gene was isolated in a mouse genetic background by three serial rounds of DNA-mediated gene transfer and fluorescence-activated cell sorting with a unique sequence probe. The gene was sublocalized to the q13.3 region of chromosome 19 by in situ hybridization. RNA transcripts of ~1.6 kb and 1.4 kb were identified in polyadenylated RNA from human myeloid leukemia cell lines using a probe from the genomic locus. Manipulation of the cloned p67 gene may provide insight into the function of its product and mechanisms regulating its expression.

MATERIALS AND METHODS

Cell lines. Low-passage NIH-3T3 cells were grown as a monolayer in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 μg/mL). Human myeloid leukemia cell lines HL-60, KG-1a, and K562 were grown in suspension cultures in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) and antibiotics as described above. NIH-3T3 cells transformed with retroviral oncogenes were grown in DMEM supplemented with 5% FBS and antibiotics. NIH-3T3 cells transfected with the neo gene were grown in DMEM with 10% FBS, antibiotics, and 400 μg/mL G418 (Geneticin, Gibco, Grand Island, NY).

DNA-mediated gene transfer. NIH-3T3 cells were cotransfected with a plasmid containing the v-fms oncogene and genomic
DNA from the human acute promyelocytic leukemia cell line HL-60 according to the calcium phosphate technique as previously described. Calcium phosphate precipitates of the plasmid and sheared genomic DNA were allowed to form at pH 6.95 for 30 minutes and then applied to monolayers of NIH-3T3 cells. After 18 hours, the cells were trypsinized and seeded into 75-cm² flasks containing DMEM with 5% FBS. After 2 weeks, transformed foci were enumerated, and the transformed cells were allowed to overgrow the nontransformed cells. The cultures were trypsinized and the cells were pooled and repassaged once before FACS with monoclonal antibodies. Genomic DNA from transformed cells was serially transfected onto NIH-3T3 cells to obtain secondary and tertiary transformants.

**Immunofluorescence and flow cytometry.** The MY9 monoclonal antibody and a control myeloma protein were obtained from Coulter Immunology, Hialeah, FL. The L1B2 and L4F3 monoclonal antibodies were the gift of Dr I. Bernstein, University of Washington, Seattle. Human leukemic cells and mouse cell transformants were stained with monoclonal antibodies by indirect immunofluorescence as previously described. Initially, the brightest 2% of the transformed NIH-3T3 cells were sorted in a sterile fashion, and foci arising from single cells were picked, grown, and analyzed by indirect immunofluorescence for binding of CD33 monoclonal antibodies.

**Southern and Northern blotting.** Genomic DNAs were digested with restriction endonucleases, separated by electrophoresis in agarose gels, and transferred to nylon membranes. RNA was extracted from human leukemia cell lines by the guanidium isothiocyanate method. Polyadenylated RNA, selected by chromatography over oligo-deoxythymidylate cellulose (Collaborative Research) was separated by electrophoresis in agarose gels containing formaldehyde and transferred to nylon membranes. Probes that recognize human repetitive sequences were prepared from sonicated, denatured human DNA self-annealed to a Cot of 2. Unique sequence probes prepared from inserts excised from plasmid vectors and purified on agarose gels, were radioiodinated with [²³¹]P] nucleotide triphosphate to a specific activity at 1 x 10⁶ dpm/µg. Molecular cloning of p67 locus. Preliminary Southern blotting analysis of DNA from independently derived tertiary transformants showed that each clone contained EcoRI fragments of 3.3-kb and 9.5-kb detected with a reiterated human DNA probe and therefore presumed to be derived from the p67 locus. Genomic DNA from a representative tertiary mouse cell transformant selected for binding of MY9 was digested to completion with EcoRI, and the restriction fragments were fractionated according to size by ultracentrifugation over a sucrose gradient. Fractions were analyzed for the presence of the characteristic 3.3-kb and 9.5-kb EcoRI fragments by Southern blotting with a human repetitive sequence probe, and the positive fractions for each fragment were separately pooled. Fractions containing the 3.3-kb fragment were ligated into λ gt10 bacteriophage arms (Stratagene, La Jolla, CA) and packaged into phage particles by use of in vitro packaging extracts (Stratagene). Fractions containing the 9.5-kb fragment were ligated into λ gt W3 bacteriophage arms (a gift of Dr Martine Roussel) and packaged into phage particles. The λ gt10 library was plated on Escherichia coli strain C600Hh + and the λ gt W3 library on LE392. Both libraries were screened with a human repetitive sequence probe, and recombinant bacteriophage containing human DNA inserts were grown by standard procedures. The inserts were subcloned into plasmid vectors and analyzed for the presence of restriction fragments that lacked repetitive human DNA sequences. The latter were used as unique sequence probes specific for the gene encoding p67.

A human genomic library prepared in the pJ88 cosmid vector was the gift of Dr W van der Ven. Colonies were plated with ampicillin on nylon membranes, replicate filters were made, and the plasmid copy number was amplified by growth in the presence of chloramphenicol. Filters were screened with a nick-translated insert probe representing unique sequences from the p67 gene. Colonies containing recombinant cosmids that annealed to the probe were isolated from replicate plates. DNA from the cosmids clones was prepared by standard procedures for isolation of plasmid DNA. Each genomic clone contained ~35 kb of human DNA. Restriction fragments were ordered by hybridizing oligonucleotide probes from the pJ88 ends of the linearized cosmid to Southern blots of partial restriction digests obtained from these linearized fragments. Recombinant DNA was handled using biosafety level 1 precautions as specified by National Institutes of Health (NIH) guidelines.

**In situ hybridization.** Radiolabeled p67-specific probes were used for in situ chromosomal hybridization to human metaphase cells prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes. A fragment from the genomic clone (1.2-kb BamHI unique sequence probe; discussed in Results section) was nick-translated in the presence of all four [³²P] labeled deoxynucleotide triphosphates to specific activities of 2 to 20 x 10⁶ dpm/µg. Hybridization and autoradiography were performed according to the procedures of LeBeau and colleagues. The slides were exposed for 10 days.

**Immunoprecipitation.** KG-la cells or transformed NIH-3T3 cells were labeled by lactoperoxidase-catalyzed cell surface iodination, and detergent lysates were immunoprecipitated with monoclonal antibodies or polyvalent antisera. Labeled proteins present in the immunoprecipitates were separated by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate (SDS) and detected by autoradiography of the dried slab gels. Mol wt was calculated by comparison to protein standards of known mol wt.

**Preparation of polyvalent antisera to p67.** Neonatal (aged 8 days) NFS mice that are syngeneic with NIH-3T3 cells were injected subcutaneously (SC) with cells from a secondary mouse cell transformant that coexpressed p67 and the product of the v-fms oncogene. Tumors formed after 2 to 3 weeks. The animals were bled, and sera were tested by indirect immunofluorescence for the presence of antibodies to p67. Positive sera were pooled and tested for the ability to recognize p67 in immunoprecipitation assays.

**RESULTS**

**DNA-mediated gene transfer.** The human gene encoding the p67 myeloid differentiation antigen was isolated in a mouse genetic background by a strategy that used DNA-mediated gene transfer followed by FACS of cells that bound a CD33 monoclonal antibody. DNA from the human acute promyelocytic leukemia cell line HL-60 was cotransfected into NIH-3T3 cells together with a plasmid containing the v-fms oncogene, which served as a dominant selectable marker by virtue of its ability to transform recipient fibroblasts. Transformed cells expressing the v-fms oncogene overview contact-inhibited, nontransformed NIH-3T3 cells in medium containing 5% FBS, and those expressing the p67 antigen were selected by FACs with CD33 monoclonal antibodies.

Flow cytometric analysis of the transformed NIH-3T3 cells with the MY9 monoclonal antibody initially failed to disclose a positive population (Fig 1A). The upper 2% of fluorescent cells were sorted, grown, and subjected to additional rounds of cell sorting with monoclonal antibody MY9. A positive population was evident after the second round of cell sorting.
Three independent, cloned tertiary mouse cell transformants that expressed p67. Transformation fragments that were not present in tertiary transformants and thus were not required to encode p67. These studies indicate that the human locus encoding p67 was included in restriction fragments containing human repeated sequences shared by the tertiary transformants.

**Derivation of a unique sequence probe for the p67 locus.** Human EcoRI fragments of 3.3 kb and the 9.5 kb present in each of the p67-positive clones were individually separated on sucrose gradients and molecularly cloned into lambda bacteriophage vectors. Screening of both libraries with the human reiterated sequence probe led to isolation of recombinant bacteriophages that contained the diagnostic EcoRI fragments. Both fragments were subcloned into plasmid vectors, mapped for sites of restriction endonuclease cleavage, and subsequently analyzed for regions lacking highly reiterated sequences. A region of the 3.3-kb EcoRI fragment contained in a 1.2-kb BamHI fragment did not anneal with the repeated sequence probe. When this 1.2-kb BamHI fragment was used as a probe in Southern blotting analysis, it specifically annealed to the 1.2-kb BamHI and 3.3-kb EcoRI fragments in normal human DNA and in the DNA from the tertiary mouse cell transformants that expressed p67. HindIII fragments of 3.4 kb and 9.5 kb were signaled with this probe in DNA from the tertiary transformants and human cells. The probe did not anneal to mouse DNA under identical conditions.

**Cloning of human p67 gene.** The unique-sequence, 1.2-kb BamHI fragment was used as a probe to screen a human genomic library prepared in the pJ88 cosmid vector. Approximately 2.5 human genome equivalents, or 200,000 recombinant colonies, were screened, and two colonies that annealed to the unique sequence probe were isolated. Hybridization of the probe to restriction endonuclease digests of DNA from both cosmids identified the expected 3.3-kb EcoRI and 1.2-kb BamHI fragments. In addition, both cosmids clones contained an EcoRI fragment that comigrated with the 9.5-kb EcoRI fragment. The restriction endonuclease map of one of the cosmids clones, designated cosmid 9, is shown in Fig 3. Mapping studies showed that the two overlapping cosmide clones differed for <10% of their length.

**Expression of p67 from cosmid genomic clones.** The cosmids clones were tested for their ability to encode p67 by transfecting them into mouse fibroblasts. DNA from each cosm was cotransfected with DNA encoding a dominant selectable marker into NIH-3T3 cells by use of the calcium phosphate technique. Parallel experiments were performed with retroviral oncogenes (v-fms, v-jun, and v-H-ras)

---

**Fig 1.** Selection of primary mouse cell transformants expressing p67 by FACS with a CD33 monoclonal antibody. In panels A through C, the fluorescence recorded using MY9 ( ) was compared with the control profile obtained using a nonimmune IgG2a myeloma protein ( ). The fluorescence histogram of NIH-3T3 cell transformants pooled immediately after transfection and stained with MY9 was not different from that obtained with the control myeloma protein (A). The 2% of cells with the brightest fluorescence were sorted, grown, and reanalyzed. After two rounds of FACS, a subpopulation of cells positive for MY9 binding was evident (B). After two more rounds of sorting, this population was predominant (C). Cloned cell lines stained positively with the monoclonal antibodies in the CD33 cluster group (D), including MY9 ( ), L1B2 ( ), and L4F3 ( ). Monoclonal antibodies from myeloid cluster groups other than CD33 gave background fluorescence histograms identical to that obtained with a control myeloma protein ( ).

In general, primary mouse cell transformants derived in this way contain in excess of 1,000 kb pairs of human DNA. Two additional serial rounds of DNA-mediated gene transfer and FACS were performed to reduce the content of human DNA in the mouse cell transformants to the minimum required to encode the p67 protein. Parallel transfections were performed with DNA from primary mouse cell transformants obtained from separate transfection experiments to obtain multiple independently derived secondary and tertiary mouse cell transformants that expressed p67. Three independent, cloned tertiary mouse cell transformants that expressed p67, as judged by their ability to bind monoclonal antibodies of the CD33 cluster group, were selected for further detailed studies.

**Human DNA content of tertiary transformants.** The genomic DNAs from the three p67-positive tertiary mouse cell transformants were analyzed for the presence of human repeated sequence families. DNA from one secondary and the three tertiary transformants was digested with BamHI, EcoRI, and HindIII and subjected to Southern blot analysis using a probe recognizing highly reiterated human DNA sequences. As shown in Fig 2, DNA from the secondary and tertiary mouse cell transformants contained discrete restriction fragments that annealed to the human repeated sequence probe. Control NIH-3T3 cell DNA did not anneal to the probe under these conditions. Positive fragments shared by each of the secondary and tertiary transformants were present after digestion with each restriction endonuclease. The secondary transformant also contained positive restriction fragments that were not present in tertiary transformants and thus were not required to encode p67. These studies indicate that the human locus encoding p67 was included in restriction fragments containing human repeated sequences shared by the tertiary transformants.
Fig 2. Southern blot analysis of human sequences in DNA from mouse cell transformants. Genomic DNAs were digested with the indicated restriction endonucleases, and the resulting fragments were separated by electrophoresis and transferred to nylon membranes. The blots were hybridized with a radiolabeled probe consisting of human highly reiterated sequence families. DNA samples were from mouse NIH-3T3 cells (M), a tertiary mouse cell transformant expressing an unrelated human myeloid differentiation antigen, gp150, (MY7-3'), and a secondary mouse cell transformant (MY9-2') and three independently derived tertiary mouse cell transformants (MY9-3') expressing p67. The migration of HindIII fragments of known sizes (in kb) from bacteriophage DNA is indicated at the left margin.

and the neo gene as dominant selectable markers. Cells selected for expression of selectable markers were tested for expression of p67 by flow cytometric analysis after staining with MY9. Significant subpopulations of cells were positive for MY9 binding in the initial flow cytometric analysis of cells selected for expression of each selectable marker. These positive subpopulations were purified by FACS to derive cells uniformly positive for expression of p67, as shown in shown in Fig 4. High levels of expression of p67 were observed regardless of whether a retroviral oncogene (v-fms, v-fes, or v-H-ras) or the neo gene was used, indicating that p67 expression was independent of the dominant selectable marker.

Production of polyclonal antisera to p67. Polyclonal antibodies to oncogene products have been produced from animals that developed tumors after inoculation with virus-transformed cells. Antisera derived in this way are frequently superior reagents for use in immunoprecipitation studies because they recognize multiple epitopes with high affinity. Neonatal NFS mice were inoculated SC with a secondary NIH-3T3 cell transformant that had highly amplified expression of p67. Because these cells were cotransformed with the v-fms oncogene, tumors developed at the injection sites after ~3 weeks. Pooled sera from tumor-bearing animals contained high-titer antibodies to p67, as determined by indirect immunofluorescence assays. The serum was tested for its ability to immunoprecipitate p67 from the human AML cell line KG-1a. Cells were labeled by lactoperoxidase-catalyzed cell surface iodination, and detergent lysates were immunoprecipitated in parallel with either pooled antisera from tumor-bearing mice or with the MY9 monoclonal antibody. Labeled proteins in the immunoprecipitates were analyzed by polyacrylamide gel electrophoresis.

As shown in Fig 5, polypeptides with electrophoretic mobilities corresponding to ~67 kd were specifically immunoprecipitated from KG-1a cells. Cells were labeled by lactoperoxidase-catalyzed cell surface iodination, and detergent lysates were immunoprecipitated in parallel with either pooled antisera from tumor-bearing mice or with the MY9 monoclonal antibody. Labeled proteins in the immunoprecipitates were analyzed by polyacrylamide gel electrophoresis.

For personal use only.on October 30, 2017. By guest www.bloodjournal.org
genomic cosmid clones. Identical results were obtained in experiments in which the 1.2-kb BamHI on chromosome 19, we performed in situ hybridization. In erythroid blood lymphocytes, there was evidence of specific bind- 

ing to the q 1 3.3 region of the long arm of chromosome 19

to DNAs from a panel of rodent x human somatic cell

nary experiments, we assigned the gene encoding p67 to

NIH-3T3 cells.

lysates from surface-iodinated control v-fms-transformed

9 and cosmid myeloma control

protein

a plasmid containing the v-fms oncogene (A), the

v-fes oncogene (B), the v-H-ras oncogene (C), and the neo gene (D), which served as dominant selectable markers.

Chromosomal localization of the p67 gene. In preliminary experiments, we assigned the gene encoding p67 to human chromosome 19 by hybridizing the p67-specific probe to DNAs from a panel of rodent x human somatic cell hybrids that contained varying complements of human chromosomes. To confirm this result and to sublocalize the gene on chromosome 19, we performed in situ hybridization. In experiments in which the 1.2-kb BamHI unique-sequence probe was annealed to metaphase chromosomes from peripheral blood lymphocytes, there was evidence of specific binding to the q13.3 region of the long arm of chromosome 19

(Fig 6). Approximately 20% of all grains present in 70 metaphases were localized to this region. Significant hybridization thought to localize to the p arm probably reflects the difficulty of distinguishing between the two arms of this metacentric chromosome in some metaphase spreads.

Identification of RNA transcripts encoding p67. Preliminary experiments showed that the 1.2-kb BamHI fragment used as a unique-sequence probe did not hybridize strongly to polyadenylated RNA from HL-60 cells, suggesting that the probe lacked exon coding sequences. Therefore, a 3.4-kb HindIII fragment present in all the p67 tertiary mouse cell transformants was used in Northern blots to identify transcripts encoded by the p67 gene in human leukemia cell lines. The probe annealed to mRNA transcrip-
ts of 1.6 kb and 1.4 kb in polyadenylated RNA from HL-60 and K562 cells, both of which express p67 on their surface. Polyadenylated RNA from the MOLT4 lymphoblastoid cell line that does not express p67 did not anneal to this probe. As shown in Fig 7, the 1.6-kb species was the predominant mRNA transcript in HL-60 cells. In contrast, the 1.4-kb species predominated in K562 cells. Larger mRNA species were detected in trace amounts in polyadenylated RNA from both cell types.

Discussion

A general strategy using DNA-mediated gene transfer and FACS with monoclonal antibodies that bind membrane proteins has previously been used to isolate the genes encoding the transferrin receptor,3132 the T4 and T8 differentiation antigens of T lymphocytes,3136 the receptor for nerve growth factor,37 and the gp150 (CD13) myeloid differentiation antigen.38 We used this approach to isolate the human gene encoding the myeloid-specific differentiation antigen p67, recognized by monoclonal antibodies of the CD33 cluster group, in a mouse genetic background. Syngeneic mice immunized with MY9-positive, v-fms-transformed mouse cell transformants developed antibodies able to precipitate p67 from human leukemia cell lines, confirming by independent criteria the validity of the gene transfer strategy. By

![Image](https://www.bloodjournal.org/)

**Fig 4.** Expression of p67 on the plasma membrane of NIH-3T3 cells transfected with the genomic cosmid clone, cosmid 9. Flow cytometric histograms obtained after binding of MY9 ( ) or a control myeloma protein ( ) to NIH-3T3 cells cotransfected with cosmid 9 and a plasmid containing the v-fms oncogene (A), the v-fes oncogene (B), the v-H-ras oncogene (C), and the neo gene (D), which served as dominant selectable markers.

![Image](https://www.bloodjournal.org/)

**Fig 5.** Immunoprecipitation of p67 encoded by the genomic clones. KG-1a cells, control v-fms transformed NIH-3T3 cells (v-fms), a secondary NIH-3T3 cell transformant selected for MY9 binding (C1), NIH-3T3 cells transfected with cosmid 1 (C1) and NIH-3T3 cells transfected with cosmid 9 (C9) were labeled by lactoperoxidase-catalyzed cell surface iodination. Detergent lysates were immunoprecipitated with MY9 or with pooled serum from tumor-bearing mice (TBS). MY7 was used as a negative control myeloma protein, and nonimmune mouse serum (NI) served as a negative control for the mouse antisera. All of the NIH-3T3 cell transformants were cotransfected with a plasmid containing the v-fms oncogene and were morphologically transformed. Labeled proteins present in the immunoprecipitates were analyzed by electrophoresis in polyacrylamide gels containing SDS. The migration of protein standards of known mol wt (in kilodaltons) is indicated at left margin.
molecular cloning of human DNA sequences from tertiary transformants, a probe for the p67 gene was obtained which, in turn, facilitated the isolation of the intact genomic locus in cosmids and its localization to the long arm of chromosome 19. The cosmid clones had all the sequences necessary to encode p67, as judged by their ability to direct the expression of a polypeptide recognized by MY9 on the surface membrane of mouse NIH-3T3 fibroblasts. These clones spanned ~35 kb of the human genome and included diagnostic EcoRI fragments containing human highly reiterated sequences shared by independently derived tertiary mouse cell transformants. Although the sequences included in the cosmid were sufficient to encode p67, the exact boundaries of the gene and its regulatory elements within the biologically active clones have not yet been established.

Various dominant selectable markers have been employed in gene transfection experiments. To date, we have generally used the v-fms oncogene as a dominant selectable marker, enabling the overgrowth of transformed cells vis à vis their nontransformed, contact-inhibited counterparts. Expression of p67 by mouse cell transfectants was also observed when the neo gene or the v-fes or v-H-ras oncogenes were used as dominant selective markers, but expression was somewhat higher when neomycin-resistant cells were selected in G418. Thus, expression of p67 from the genomic clones was independent of the dominant selectable marker used.

Unlike other protein differentiation antigens present on human myeloid cells, expression of p67 is normally limited to cells of the myeloid lineage. The basis for efficient expression of p67 by mouse fibroblasts transfected with the genomic clones remains unclear, but similar digressions from tissue-specific expression have been observed in transfection experiments involving the genes encoding T-lymphocyte differentiation antigens and the nerve growth factor receptor.

Two major mRNA transcripts were detected in polyadenylated RNA from human myeloid leukemia cell lines that bind MY9. In the HL-60 acute promyelocytic leukemia cell line, a 1.6-kb species predominated, whereas a 1.4-kb species was preferentially expressed by the K562 erythroleukemia cell line. The presence of alternative transcripts could result from differential splicing, possibly leading to heterogeneity in mRNA stability or subtle differences in the coding sequences for the p67 polypeptides expressed by myeloid precursor cells at various stages of maturation and differentiation. The complexity of these mRNAs would be sufficient to encode a protein of 67-Kd. However, the formal demonstration that these are bona fide p67 mRNAs awaits analysis of cDNA clones and in vitro translation.

The function of p67 in immature myeloid cells is unknown. One possibility is a physiologic role restricted to myeloid
progenitors, since terminally differentiated myeloid cells, lymphoid cells, and nonhematopoietic cells have not been found to express this protein. The p67 probes can be used to obtain complementary DNA clones, which would facilitate the determination of the predicted primary structure of the protein, and might provide insight into its topology and function. Studies of the regulatory sequences of the gene may also reveal mechanisms that confer myeloid-specific expression of p67 in human cells.

REFERENCES

25. LeBeau MM, Westbrook CA, Diaz MO, Rowe JD: c-src is consistently conserved in the chromosomal deletion (20q) observed in myeloid disorders. Proc Natl Acad Sci USA 82:6692, 1985
31. Kuhn LC, McClelland A, Ruddle FH: Gene transfer, expres-
Molecular cloning, expression, and chromosomal localization of a human gene encoding the CD33 myeloid differentiation antigen

SC Peiper, RA Ashmun and AT Look