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cDNA cloning of a human mRNA preferentially expressed in hematopoietic cells and with homology to a GDP-dissociation inhibitor for the rho GTP-binding proteins

(subtractive libraries/hematopoietic cDNA/guanine nucleotide exchange/differentiation/embryonic stem cell)

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ABSTRACT **We have identified the mRNA for a human gene, denoted D4, which is expressed at very high levels in hematopoietic cell lines and in normal cells of lymphoid and myeloid origin. The 1.5-kb transcript is absent or detectable only at low levels in nonhematopoietic tissues. D4 encodes a 201-amino acid protein with homology to rhoGDI, an inhibitor of GDP dissociation for the ras-homologous protein rho. D4 might function also as a regulator of guanine nucleotide exchange for small GTP-binding proteins. A homologous transcript of similar size is also preferentially expressed in murine hematopoietic tissues. When totipotent murine embryonic stem cells develop *in vitro* into hematopoietic cells, the gene is activated with the onset of hematopoiesis. When hematopoietic cell lines are induced to differentiate, the expression of D4 is modulated. Thus, D4 appears to be a developmentally regulated gene. Its preferential expression in hematopoietic cells indicates that D4 likely plays some significant role in the growth and differentiation processes of hematopoietic cells. This significance is underscored by increasing evidence for the involvement of regulators of G proteins in clinical diseases.**

In the hematopoietic system, a common pluripotent stem cell gives rise to at least eight distinct lineages. While cells of each lineage appear to have strictly defined characteristics and functions, a considerable plasticity in lineage specificity at the cellular and molecular level has been observed. For example, leukemic cells had been found to express molecular markers of more than one lineage in the same cell (1–4). It is unclear at the moment whether this reflects a distortion of the genetic mechanism of differentiation (2) or the normal differentiation process of stem cells (5). A more dramatic example of this plasticity is the demonstration of the conversion of pre-B cells, which had undergone VDJ rearrangement, into macrophages (6, 7). These dedifferentiated cells continue to maintain their immunoglobulin gene rearrangements but morphologically and functionally behave as macrophages. It is remarkable that cells with a stably rearranged genome can convert into cells of a completely different lineage. These observations suggest that beneath the diversity some or all hematopoietic lineages continue to remain closely related. Hematopoietic cells may then be viewed not as a system of distinctly differentiated cells but as a family of related cells among which common features may be detected that distinguish them from other tissues. We surmise that there are molecules that regulate molecular events unique to all hematopoietic cells and that these molecules are likely to be important even after commitment into specific lineages. We therefore attempted to identify genes that are either preferentially or specifically expressed in hematopoietic cells. By differential screening of a subtractive hematopoietic cell cDNA library with subtractive probes, we have identified and isolated the cDNA clones of several genes manifesting these characteristics. In this communication, we describe the molecular structure and expression of one of these clones, which we have denoted D4. Transcripts for D4 are expressed preferentially at a very high level in hematopoietic cells of all lineages. Most interestingly, D4 encodes a protein with homology to a recently described bovine rhoGDI (8), a GDP-dissociation inhibitor (GDI) for the rho family of GTP-binding proteins.§

MATERIALS AND METHODS

Cell Lines. Hematopoietic cell lines used in this study included pluripotent (DU528, K562), erythroleukemia (HEL, OCIR), monoblastic (U937), myeloblastic (KG-1), promyelocytic (HL60), T-cell lymphoma and leukemia (LY-17, Molt-4), myeloma (OCI-My1), megakaryocytic (Dami), and murine erythroleukemia (MEL) cells. Nonhematopoietic cell lines used were bone marrow stromal (BS-1), hepatoma (HepG2), lung cancer (Calu-1), cervical cancer (HeLa), melanoma (HS294, SKMEL), skeletal muscle (HuSk), neuroblastoma (SKNSH), and skin fibroblasts. All cell lines were maintained in Iscove's modified Dulbecco's medium supplemented with 10% fetal bovine serum (FBS) and 1 mM L-glutamine, except for DU528, in which horse serum replaced FBS.

Construction of Subtractive cDNA Libraries. Three hematopoietic cell lines (K562, KG-1, and DU528) and a nonhematopoietic human bone marrow stromal line (BS-1) were used in construction of subtractive cDNA libraries and generation of probes for differential screening. cDNAs made from the four cell lines were hybridized with excess BS-1 mRNA. Unhybridized cDNAs were separated by using a cDNA·mRNA hybridization technique as described by Sive and St. John (9). mRNAs of BS-1 were biotinylated with photobiotin acetate (Invitrogen, San Diego) following a protocol recommended by the vendor. cDNA biotinylated mRNA hybrids and excess biotinylated mRNAs were removed by treatment with streptavidin (Invitrogen) followed by phenol extraction, leaving unhybridized cDNAs in the aqueous phase. Two rounds of subtractive hybridization were performed. The resulting unhybridized cDNAs were used to construct four libraries (K562/BS-1, KG-1/BS-1,

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Abbreviations: G protein, GTP-binding protein; ES cell, embryonic stem cell; FBS, fetal bovine serum; PMA, phorbol 12-myristate 13-acetate; DMSO, dimethyl sulfoxide.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. L07916).

DU528/BS-1, and BS-1/BS-1 as described by Caput *et al.* (10), using the PT3T719U multiphagemid vector (Pharmacia). cDNAs were cloned directionally into the *Pst* I and *Bam*HI sites.

Generation of DNA Probes from Subtractive Libraries. Recombinant DNA was purified from the KG-1/BS-1, DU528/BS-1, and BS-1/BS-1 subtractive libraries. cDNA inserts were released by restriction enzymes *Pst* I and *Bam*HI and labeled with ^{32}P by multiprime labeling (Pharmacia).

***In Vitro* Differentiation of Murine Embryonic Stem (ES) Cells.** The CCE ES cell line was maintained and passaged as described (11) in the presence of leukemic inhibitory factor (Genetics Institute, Cambridge, MA). Differentiation of totipotent ES cells into hematopoietic cells was studied by an *in vitro* assay developed in our laboratory. Similar methods have been described by others (12). Single cell suspensions of the CCE cell line were cultured in non-tissue culture 35-mm dishes (Fisher) with a mixture containing 0.9% methylcellulose (Fluka), 20% FBS (HyClone), 1% bovine serum albumin, erythropoietin (2 units/ml) (D. Worchowski, Pennsylvania State University, College Park), stem cell factor (50 ng/ml) (Amgen Biologicals), and interleukin 1 (200–400 units/ml) (Hoffmann–La Roche) at a cell concentration of 1000–2000 cells per ml. Incubation was carried out in a humidified atmosphere at 37°C and colonies were examined and collected on different days after initiation of culture by pooling colonies from several dishes.

Induction of Cell Lines. The cell lines U937, HL60, and MEL were grown to a concentration of 5×10^5 cells per ml before initiation of induction as follows: U937 with 50 nM phorbol 12-myristate 13-acetate (PMA; Sigma), HL60 with PMA or 4% dimethyl sulfoxide (DMSO); MEL with 1.5% DMSO.

General Methods. Standard methods were carried out as described by Sambrook *et al.* (13). RNA was isolated by the guanidinium isothiocyanate/CsCl procedure. DNA sequencing was done by the dideoxynucleotide chain-termination technique (14) after subcloning appropriate DNA fragments into M13.

RESULTS

Construction of Subtractive Libraries. We generated cDNA libraries of hematopoietic cell lines that are enriched for cDNAs of mRNA preferentially or uniquely expressed in hematopoietic cells. This was achieved by subtracting cDNAs of the hematopoietic cell lines with mRNAs from a nonhematopoietic cell line. The hematopoietic cell lines were chosen on the basis of their primitive stem cell characteristics. K562 cells express markers for erythroid, granulocytic, and megakaryocytic lineages (2). KG-1 cells, a myeloblastic line, express the CD34 surface antigen (15), which is a marker for multipotent stem cells. DU528 cells, derived from a patient with leukemia, had been shown to be capable of differentiating into both lymphoid and myeloid lineages (16). Since the objective of subtraction was to reduce the complexity of the libraries, the choice of the nonhematopoietic mRNAs used for subtraction was made arbitrarily. We chose a human bone marrow stromal-derived line, BS-1 (17), established in our own laboratory. A normalized stromal BS-1 cDNA library enriched for low-frequency molecular species was also constructed by using stromal cDNAs obtained after subtracting twice with stromal mRNAs.

Differential Screening and Identification of Hematopoietic Specific Clones. The established libraries became a ready source of probes. cDNA inserts from two hematopoietic (DU528 and KG-1) and the BS-1 subtractive libraries were released and purified. The three probes were then used to hybridize against triplicates of the K562 library. Colonies

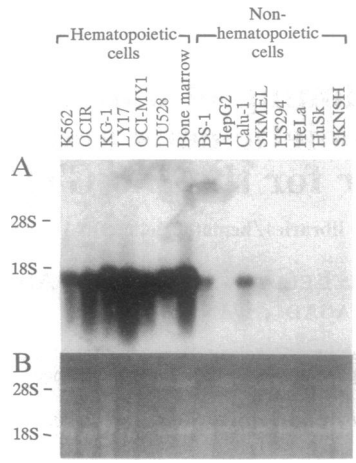

FIG. 1. Northern blot analysis of D4 mRNA in human hematopoietic and nonhematopoietic cell lines. (*A*) Fifteen micrograms of total RNA was resolved on a denaturing agarose gel, transferred to Hybond-N filters (Amersham), hybridized with ^{32}P-labeled D4 cDNA probe, and washed with 0.2× standard saline citrate at 65°C before autoradiography for 24 hr at −80°C. (*B*) Ethidium bromide-stained gel of the RNA in *A* to monitor integrity of samples and constancy of loading.

hybridizing with the hematopoietic probes (DU528 and KG-1) and not with the BS-1 probe were isolated and expanded. DNAs from these clones were extracted and used as individual probes to examine Northern blots consisting of a panel of total RNAs from hematopoietic and nonhematopoietic cell lines. The patterns of expression allowed us to identify cDNA clones of different mRNAs that are either preferentially or specifically expressed in hematopoietic cells.

Identification of Clone D4. Clone D4 contains an insert that detects a 1.5-kb transcript present in great abundance in myeloid and lymphoid hematopoietic cell lines (Fig. 1; see also Fig. 6). In contrast, nonhematopoietic cell lines either do not express the transcript (HepG2, HeLa) or do so at a much lower level. Only cell lines of bone marrow stroma (BS-1) and lung (Calu-1) expressed a comparable level of D4, but, even in these cells, the levels of D4 expressed were consistently lower than in hematopoietic cells.

Expression of D4 in Normal Human and Murine Tissues. Total RNAs from various normal human tissues were examined to compare with the results from cell lines (Fig. 2). Bone marrow cells and peripheral blood T lymphocytes expressed a high level of the transcript. In contrast, a weaker signal was seen in the lung while a significantly lower level was seen in other nonhematopoietic tissues, including kidney, liver, adrenal, and muscle. A barely detectable signal was seen in brain cells and no transcript was detected in skin fibroblasts. Examination of RNAs from murine tissues with the human

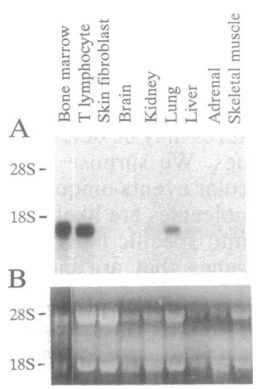

FIG. 2. Northern blot analysis of D4 in 15-μg RNA samples extracted from normal human tissues. Human RNAs (except bone marrow, T lymphocytes, and skin fibroblasts) were the gift of B. Seizinger (Massachusetts General Hospital, Boston). (*A*) Autoradiograph of membrane. (*B*) Ethidium bromide-stained gel.

cDNA also detected a strongly hybridizing 1.5-kb transcript expressed preferentially in hematopoietic tissues including bone marrow cells, spleen, thymus, and lymph nodes (data not shown).

Molecular Characterization of D4. Fig. 3 shows the nucleotide sequence of the D4 cDNA and the predicted amino acid sequence. The cDNA contains an open reading frame encoding a protein of 201 amino acids with a calculated molecular mass of 23 kDa. This is consistent with our preliminary observation of a 23- to 24-kDa protein obtained by expression of the cDNA in bacteria. Analysis with the Kyte–Doolittle algorithm (18) revealed the protein to be markedly hydrophilic in its overall characteristic with no hydrophobic domain indicative of a membrane-spanning region (data not shown). A search of the GenBank data base, using the FASTA program (19), revealed that clone D4 bears homology to the bovine rhoGDI cDNA (8). They share 60% identity at the nucleic acid level, while the predicted protein sequences are 67% identical (Fig. 4). The homology is distributed throughout the cDNA. The greatest divergence is seen within the first 25 amino acids, where identical residues dropped to 16%. No significant homology to other genes, particularly other regulators of guanine nucleotide binding (G) proteins, was identified.

Expression of D4 During Differentiation of ES Cells. To examine the expression of D4 during the earliest events of hematopoiesis, we studied the regulation of its expression during the transition of uncommited embryonic cells into hematopoietic cells. We used an *in vitro* system that we and others (12, 20) developed whereby murine ES cells, cultured in semisolid conditions, undergo development from single cells into large colonies containing hematopoietic cells of the erythroid, granulocytic, and megakaryocytic lineages. Colonies on different days after culture were harvested and total RNA was extracted. In a typical experiment, colonies at days 9–10 will display hematopoiesis most easily recognized under inverted light microscopy by a dense ring of erythroid cells mixed with nonerythroid cells or a central core of blood island. Fig. 5 shows a Northern blot of a panel of RNAs from colonies at days 2, 4, 6, 8, 10, and 12. The filter was probed for D4 and mRNA of several other genes indicative of development along different hematopoietic lineages; embryonic globin βh1 for primitive erythropoiesis (21), Mac-I (an integrin cell surface antigen) for mature granulocytes and macrophages (22), and the macrophage mannose-receptor MøMR, specifically for macrophage lineage (23). On day 6, before erythroid cells were recognizable in the colonies, transcripts for βh1 globin were clearly detected. MøMR and D4 transcripts were first detectable between days 6 and 8. Mac-I transcripts were first detected on day 10 and increased strongly by day 12.

Expression of D4 During Induced Differentiation of Hematopoietic Cell Lines. As a further evaluation of the biological function of D4 in hematopoietic cells, we searched for evidence of modulation of its expression during differentiation. Several cell lines representative of various lineages were used in induction experiments. Fig. 6 shows that different lineages are modulated differently as a result of induction. In U937, a myelomonocytic cell line, the induction of differentiation into macrophages by PMA caused a down-regulation of D4 so that by 48 hr only a very low level of D4 was detected (Fig. 6*a*). This was in contrast to the activation and up-regulation of CD11b, the human homologue of Mac-I. When HL60 cells were induced to differentiate into neutrophils (monitored by CD11b expression), no noticeable change in the level of D4 (Fig. 6*b*) was seen. However, when HL60 cells

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                                                5'AGTACTCAGAAGTC   14

    AGAGTTGAGAGACAGAGGCACCCCGGACAG  AGACGTGAAGCACTGAATAAATAGATCAGA   74

    ATGACTGAAAAAGCCCCAGAGCCACATGTG  GAGGAGGATGACGATGATGAGCTGGACAGC  134
1   MetThrGluLysAlaProGluProHisVal  GluGluAspAspAspAspGluLeuAspSer

    AAGCTCAATTATAAGCCTCCACCACAGAAG  TCCCTGAAAGAGCTGCAGGAAATGGACAAA  194
21  LysLeuAsnTyrLysProProProGlnLys  SerLeuLysGluLeuGlnGluMetAspLys

    GATGATGAGAGTCTAATTAAGTACAAGAAA  ACGCTGCTGGGAGATGGTCCTGTGGTGACA  254
41  AspAspGluSerLeuIleLysTyrLysLys  ThrLeuLeuGlyAspGlyProValValThr

    GATCCGAAAGCCCCCAATGTCGTTGTCACC  CGGCTCACCCTGGTTTGTGAGAGTGCCCCG  314
61  AspProLysAlaProAsnValValValThr  ArgLeuThrLeuValCysGluSerAlaPro

    GGACCAATCACCATGGACCTTACTGGAGAT  CTGGAAGCCCTCAAAAAGGAAACCATTGTG  374
81  GlyProIleThrMetAspLeuThrGlyAsp  LeuGluAlaLeuLysLysGluThrIleVal

    TTAAAGGAAGGTTCTGAATATAGAGTCAAA  ATTCACTTCAAAGTGAACAGGGATATTGTG  434
101 LeuLysGluIleGlySerGluTyrArgVal  LysHisPheLysValAsnArgAspIleVal

    TCAGGCCTGAAATACGTTCAGCACACCTAC  AGGACTGGGGTGAAAGTGGATAAAGCAACA  494
121 SerGlyLeuLysTyrValGlnHisThrTyr  ArgThrGlyValLysValAspLysAlaThr

    TTTATGGTTGGCAGCTATGGACCTCGGCCT  GAGGAGTATGAGTTCCTCACTCCAGTTGAG  554
141 PheMetValGlySerTyrGlyProArgPro  GluGluTyrGluPheLeuThrProValGlu

    GAGGCTCCCAAGGGCATGCTGGCCCAAGAC  ACGTACCACAACAAGTCCTTCTTCACCGAC  614
161 GluAlaProLysGlyMetLeuAlaGlnAsp  ThrTyrHisAsnLysSerPhePheThrAsp

    GATGACAAGCAAGACCACCTCAGCTGGGAG  TGGAACCTGTCGATTAAGAAGGAGTGGACA  674
181 AspAspLysGlnAspHisLeuSerTrpGlu  TrpAsnLeuSerIleLysLysGluTrpThr

    GAATGAATGCATCCACCCCTTTCCCCACCC  TTGCCACCTGGAAGAATTCTCTCAGGCGTG  734
201 GluEnd

    TTCAGCACCCTGTCCCTCCTCCCTGTCCAC  AGCTGGGTCCCTCTTCAACACTGCCACATT  794
    TCCTTATTGATGCATCTTTTCCCACCCTGT  CACTCAACGTGGTCCCTAGAACAAGAGGCT  854
    TAAAACCGGGCTTTCACCCAACCTGCTCCC  TCTGATCCTCCATCAGGGCCAGATCTTCCA  914
    CGTCTCCATCTCAGTACACAATCATTTAAT  ATTTCCCTGTCTTACCCCTATTCAAGCAAT  974
    TAGAGGCCAGAAAATGGGCAAATTATCACT  AACAGGTCTTTGACTCAGGTTCCAGTAGTT 1034
    CATTCTAATGCCTAGATTCTTTTGTGGTTG  TTGCTGGCCCAATGAGTCCCTAGTCACATC 1094
    CCCTGCCAGAGGGAGTTCTTCTTTTGTGAG  AGACACTGTAAACGACACAAGAGAACAAGA 1154
    ATAAAACAATAACTGTGAAAAAAAAA 3'   1180
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FIG. 3. Nucleotide and deduced amino acid sequence of clone D4. Amino acid sequence of the longest open reading frame is shown numbered from the presumed initiating methionine. TGA stop codon (End) is followed by a 3′ untranslated region containing an AATAAA poly(A) addition sequence (underlined). In-frame upstream stop codon TAG at position 66 is boxed. Consensus N-glycosylation sites (Asn-Xaa-Ser/Thr) are underlined by an open bar.

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D4          MTEKAPEPHVEED...DDDELDSKLNYKPPPQKSLKELQEMDKDDESLIK  47
            |*|  | *   *   ***| *  *|||||*|||* |*||*|||||||| |
Bovine      MAEQEPTAEQLAQIAAENEEDEHSVNYKPPAQKSIQEIQELDKDDESLRK  50
rhoGDI

D4          YKKTLLGDGPVVTDPKAPNVVVTRLTLVCESAPGPITMDLTGDLEALKKE  97
            || *|||  *| *||  |||||||||||| *||||* *|||||||* ||*
Bovine      YKEALLGRVAVSADPNVPNVVVTRLTLVCSTAPGPLELDLTGDLESFKKQ  100
rhoGDI

D4          TIVLKEGSEYRVKIHFKVNRDIVSGLKYVQHTYRTGVKVDKATFMVGSYG  147
            * ||||| |||*|| |*|||*||||*||*||||| |||*||* *||||||
Bovine      SFVLKEGVEYRIKISFRVNREIVSGMKYIQHTYRKGVKIDKTDYMVGSYG  150
rhoGDI

D4          PRPEEYEFLTPVEEAPKGMLAQDTYHNKSFFTDDDKQDHLSWEWNLSIKK  197
            ||*|||||||*|||||||||  *|  || |||||* |||||||||*|||
Bovine      PRAEEYEFLTPMEEAPKGMLARGSYNIKSRFTDDDRTDHLSWEWNLTIKK  200
rhoGDI

D4          EWTE      201
            || *
Bovine      EWKD      204
rhoGDI
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FIG. 4. Comparison of human D4 with bovine rhoGDI protein sequences using the program of Pearson and Lipman (19). Vertical lines indicate identical matches and asterisks indicate conservative changes. Gaps introduced to maximize alignment are indicated by dots.

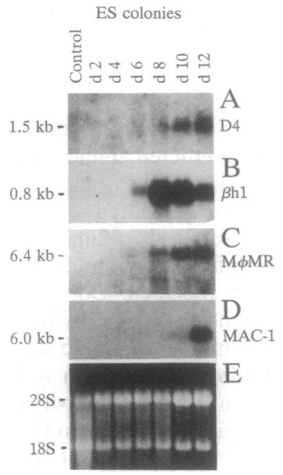

FIG. 5. Northern blot analysis of D4 (*A*), embryonic globin βh1, (*B*), murine macrophage mannose receptor MφMR (*C*), and MAC-1 (*D*) in RNAs of ES cell colonies pooled and collected after different days in culture for hematopoietic differentiation. The same blot was probed sequentially (*A–D*). (*E*) Ethidium bromide-stained gel.

were induced to differentiate into macrophages by PMA, down-regulation of the transcript was observed as in U937 cells (data not shown). When the murine erythroleukemia cell line MEL was induced to undergo terminal differentiation by DMSO, a strong and rapid up-regulation of D4 was evident within the first 2 hr followed by gradual down-regulation to base-line level by 48 hr (Fig. 6*c*). Differentiation of MEL cells was indicated by >80% benzidine-positive cells and the appearance of adult β-globin transcripts (data not shown).

DISCUSSION

Examination of both cell lines and normal tissues showed that D4 is expressed at a high level only in hematopoietic cells and is well conserved across species. This suggests that D4 has some unique functional significance in hematopoietic cells.

Supportive evidence was obtained by examining the expression of D4 during early hematopoiesis in murine ES cells and during differentiation in inducible hematopoietic cell lines. ES cells are totipotent embryonal cells capable of developing into every tissue type of the animal (24). The CCE cell line used in our experiments was maintained in liquid culture as undifferentiated cells in the presence of leukemic inhibitory factor. Using the *in vitro* methylcellulose culture system described, individual cells developed into mixed colonies containing erythrocytes, granulocytes, and megakaryocytes. The expression of globin genes in these colonies mimics the temporal pattern of embryonic, fetal, and adult globin gene activation in developing embryos (12, 25). Colonies from such *in vitro* cultures of ES cells, containing targeted disruption of the *GATA-1* gene, displayed an abrogation of erythroid cell development similar to what was observed in transgenic animals generated by the same mutated ES cells (26). Therefore, the assay provides a useful alternative to *in vivo* studies of the expression and function of specific genes during hematopoietic cell development. We compared the kinetics of D4 expression in these colonies to the expression of three other hematopoietic specific genes—βh1, MøMR, and Mac-I—as markers of embryonic erythroid, macrophage, and mature granulocytic differentiation. βh1 transcripts were detected earliest, followed by MøMR and D4. Mac-I was detected in later stage colonies, reflecting the emergence of mature neutrophils and macrophages in the colonies. Therefore, it appears that as early hematopoietic cells develop from embryonic cells, D4 is activated. The difference between embryonic hematopoiesis and definitive adult hematopoiesis is still unclear. That D4 transcripts were seen after the initiation of embryonal globin transcription suggests that D4 might not be relevant during embryonic hematopoiesis or at least not during embryonic erythropoiesis.

Hematopoietic cell lines induced to undergo differentiation revealed markedly different regulation of D4 expression. During differentiation into macrophages, D4 transcripts became significantly down-regulated. In contrast, differentiation into neutrophils showed no obvious change in the level of D4 expressed while differentiation along the erythroid lineage caused a very early transient increase followed by a decrease to base-line level.

Taken together, these results suggest that D4 is involved in the regulatory processes of adult hematopoiesis and that it may function differently in different lineages.

Molecular analysis revealed that, at the protein level, D4 shared 67% homology with bovine rhoGDI (8). We have cloned murine cDNA for D4 and the predicted protein is 89% identical to the human protein (unpublished data). Human rhoGDI has been most recently described and is 93% identical to bovine rhoGDI (27). D4 and rhoGDI are not homologues but are most likely members of a family of related genes. We do not have any information yet about the biochemical function of D4. Its homology to rhoGDI suggests possible related activities. rhoGDI inhibits dissociation of guanine nucleotide GDP from the ras-homologous protein rho and is the rate-limiting regulator of active GTP-bound rho (8). It is likely that as one of its functions D4 acts as a regulator of guanine nucleotide exchange for a known or as yet unknown ras-related small G protein. Little is currently known about the function of the family of small G proteins, but several lines of evidence indicate that they are involved in controlling such diverse cellular functions as cell division, differentiation, cytoskeletal organization, and intracellular vesicle transport and secretion (28). Regulators of the active state of G proteins are therefore critical molecules. This importance is underscored by increasing evidence implicating them in clinically important diseases. The *dbl* protooncogene, identified initially as an oncogene in a human B-cell lymphoma, is a guanine nucleotide exchange factor for the ras-related protein, CDC42Hs (29). *bcr*, the breakpoint cluster region gene involved in chronic myeloid leukemia, encodes a multifunctional protein that includes a dbl-homologous domain (30) and a GTPase activating protein (GAP) for $p21^{rac}$ (31). Neurofibrin (NF1), the neurofibromatosis type 1 susceptibility gene, is a GAP protein for $p21^{ras}$.

Preferential expression of D4 in blood cells is indicative of a gene with unique and functional significance in hematopoietic cells. It is interesting to note that *vav* (33), a protooncogene expressed specifically in all hematopoietic lineages, contains a region sharing homologies with the GDP/GTP exchange domain of dbl, CDC24, and bcr (34). These proteins

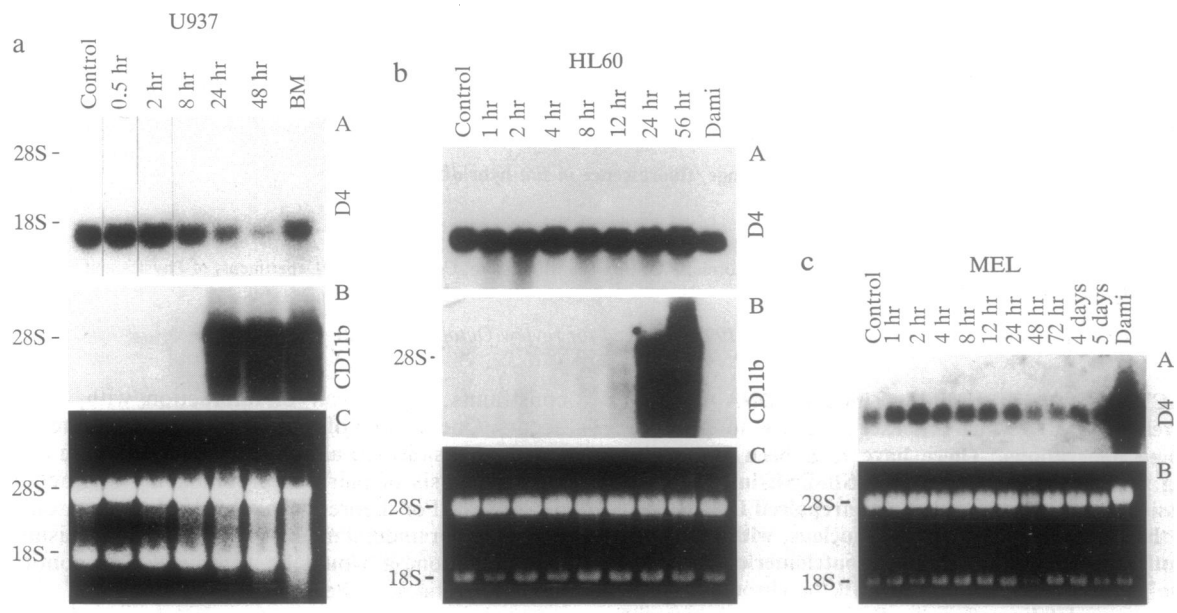

FIG. 6. Northern blot analysis of hematopoietic cell lines at different times after initiation of induction. BM, bone marrow. (*a*) U937 cells induced with PMA. The same blot was probed sequentially with D4 (*A*) and with CD11b (*B*). (*b*) HL60 cells induced with DMSO. (*c*) MEL cells induced with DMSO.

may represent a family of positive regulators acting as catalysts for dissociation of GDP from G proteins in different tissues. It is very likely that a group of GDP-dissociation inhibitors, such as rhoGDI, function as negative regulators of G-protein activation. Determination of the biochemical action of D4 is therefore of great interest.

C.N.A. and J.-M.L. made equal contributions to this work. We thank Dr. J. Kurtzberg (Duke Univ., Durham, NC) for cell line DU528; Dr. H. A. Messner (Univ. of Toronto, Toronto) for cell lines OCI-R, LY-17, and OCI-My1; Dr. I. Saigo (Beth Israel Hospital, Boston) for cell line HuSk; and Dr. S. H. Orkin (Children's Hospital, Boston) for the CCE ES cell line. We also thank Dr. R. A. B. Ezekowitz (Children's Hospital) for the murine MøMR probe, Dr. D. G. Tenen (Beth Israel Hospital) for the CD11b and Mac-I probe, and Dr. M. Baron (Harvard Medical School) for the βh1 probe. This work was supported by National Institutes of Health Grant DK 44099-02.

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