MOLECULAR AND CELLULAR BIOLOGY, Aug. 1994, p. 5099–5107
0270-7306/94/$04.00+0

Vol. 14, No. 8

Molecular Cloning of a Novel Human cDNA Encoding a Zinc Finger Protein That Binds to the Interleukin-3 Promoter

NAOKO KOYANO-NAKAGAWA,[1] JUNJI NISHIDA,[2]† DAVID BALDWIN,[2]
KEN-ICHI ARAI,[1,2] AND TAKASHI YOKOTA[1,2]*

Department of Molecular and Developmental Biology, The Institute of Medical Science, The University of Tokyo, Minato-ku, Tokyo 108, Japan,[1] and Department of Molecular Biology, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, California 94304[2]

Received 30 December 1993/Returned for modification 2 February 1994/Accepted 2 May 1994

The CT/GC-rich region (−76 to −47) is one transcriptional regulatory region of the interleukin-3 (IL-3) gene which confers basic transcriptional activity and responds to *trans*-activation by human T-cell leukemia virus type I-encoded Tax. We isolated three types of cDNAs encoding Cys_2/His_2-type zinc finger proteins that bind to this region. Two were identical to known transcription factors, EGR1 and EGR2, and the other clone, named DB1, encoded a novel protein of 516 amino acids with six zinc finger motifs. DB1 mRNA was present in human tissues, ubiquitously. Two constitutive transcripts of 4.0 and 4.8 kb in length were present in Jurkat cells. Electrophoretic mobility shift assay, with specific antibodies, showed that DB1 constitutively binds to this region whereas EGR1 binds in a T-cell activation-dependent manner. Overexpression of DB1 in Jurkat cells had no detectable effect on the transcription activity of the IL-3 promoter, in a transient-transfection assay. EGR1 and EGR2 increased IL-3 promoter activity when the transfected cells were stimulated with phorbol-12-myristate-13-acetate and A23187. When DB1 was cotransfected with a Tax expression vector, transcription activity of the IL-3 promoter induced by Tax was significantly increased, while EGR1 and EGR2 were without effect. These results suggest that EGR1 has a role in inducible transcription of the IL-3 gene, while DB1 sustains basal transcriptional activity and also cooperates with Tax to activate the IL-3 promoter.

Interleukin-3 (IL-3) is a hemopoietic growth factor supporting the proliferation and differentiation of early precursors of the myeloid, erythroid, monocytic, and megakaryocytic cell lines (36). The human IL-3 gene is located on chromosome 5, and its transcription is induced in activated T cells. IL-3, expressed in a tissue-specific manner, is restricted to antigen-stimulated helper T cells, natural killer (NK) cells, and immunoglobulin E (IgE) cross-linked mast cells (26, 32, 42).

T-cell activation can be mimicked by treatment with phorbol-12-myristate-13-acetate (PMA) and calcium ionophore (A23187). PMA and A23187 treatment leads to activation of various transcription factors as well as to production of lymphokines, including IL-2, IL-3, and granulocyte-macrophage colony-stimulating factor (39). The 5′ upstream regulatory region of the human IL-3 gene has been well studied, and elements that account for inducible as well as constitutive expression of the gene have been identified (6, 15, 21, 25, 28, 35). The AP1 binding site (−301 to −295) and the NF-IL3-A/ACT-1 binding site (−156 to −147) were found to be stimulation-responsive elements in Jurkat, MLA144, and primary human T cells. Transcription of the IL-3 gene is also negatively regulated by NIP (−271 to −250), an element which is recognized by a negatively acting protein complex. We previously identified the CT/GC-rich region, at position −76 to −47, and found it to be required for basal promoter activity of the human IL-3 gene in vitro (25) and also for basal transcriptional activities on heterologous promoters in vivo (15). We also reported that the CT/GC-rich region serves as a responding element for human T-cell leukemia virus type I-encoded Tax (25). Thus, the CT/GC-rich region is one of the essential elements for the IL-3 promoter. Electrophoretic mobility shift assay (EMSA) with the CT/GC-rich region gave rise to several DNA-protein complexes, thereby indicating that the DNA-binding protein(s) that bind to this region is present in the nuclear fraction of Jurkat cells (25). Mutation analysis indicated a good correlation between specific DNA-binding activity and in vitro transcriptional activity (25).

We report here the cloning and characterization of cDNAs encoding binding proteins for the CT/GC-rich region. One of the cloned genes encoded a novel zinc finger protein, DB1. The possible involvement of DB1 protein as well as EGR1 and EGR2 in the transcription of the IL-3 gene is discussed.

MATERIALS AND METHODS

cDNA libraries. The λgt11 expression library of stimulated Jurkat cells was provided by Kyoko Yokota and Naoko Arai (DNAX Research Institute). It is a unidirectional cDNA library constructed by using the Riboclone cDNA synthesis system and λgt11 *Eco*RI-*Not*I arms (Promega).

Southwestern (DNA-protein) screening. The method of Southwestern screening we used has been described elsewhere (34). An oligonucleotide with three tandem repeats of the recognition sequence at positions −76 to −50 (CCTCCCCC CGCCTTGCCCCTCCCCCCGCCTTGCCCCTCCCCC CGCCTTGCC; underlined sequence is one unit) was synthesized and labeled by Klenow reaction with [α-^{32}P]dGTP (Amersham). IL-3 upstream monomer probe covering positions −100 to −39 was also synthesized and was used to confirm the binding. After partial sequencing of the positive clones, the fragments were labeled and used as probes to screen a cDNA

* Corresponding author. Mailing address: Department of Molecular and Developmental Biology, The Institute of Medical Science, The University of Tokyo (IMSUT), 4-6-1 Shirokanedai, Minato-ku, Tokyo 108, Japan. Phone: 81-3-5449-5661. Fax: 81-3-5449-5424. Electronic mail address: tyokota@ims.u-tokyo.ac.jp.

† Present address: Department of Internal Medicine, Teikyo University, Itabashi-ku, Tokyo 173, Japan.

library of activated Jurkat cells in order to obtain full-length clones.

Sequencing and homology search. Sequencing was done after subcloning into pBluescript II KS (−) (Stratagene) with Sequenase (U.S. Biochemicals). The homology search was done with the FASTA and TFASTA programs of the GenBank (release 79.0) and EMBL (release 35.0) databases (31).

Plasmids and oligonucleotides. Oligonucleotides used for EMSA are listed below. Mutated nucleotides are underlined.

CT/GC-wild (wild type) 5′-GATCCCTCCCCCCGCCTTGCCCGGGGTTGTGGGA-3′
3′-GGAGGGGGGCGGAACGGGCCCCAACACCCTCTAG-5′

CT/GC-mut1 5′-GATCCTAGACCCCGCCTTGCCCGGGGTTGTGGGA-3′
3′-GATCTGGGGCGGAACGGGCCCCAACACCCTCTAG-5′

CT/GC-mut2 5′-GATCCCTCCTCTAGACTTGCCCGGGGTTGTGGGA-3′
3′-GGAGGAGATCTGAACGGGCCCCAACACCCACTAG-5′

CT/GC-mut3 5′-GATCCCTCCCCCCCGCTCTAGACGGGGTTGTGGGC-3′
3′-GGAGGGGGGCGAGATCTGCCCCAACACCCGCTAG-5′

CT/GC-mut4 5′-GATCCCTCCCCCCGCCTTGCCTCTAGATGTGGGC-3′
3′-GGAGGGGGGCGGAACGGAGATCTACACCCGCTAG-5′

CT/GC-mut5 5′-GATCCCTCCCCCCGCCTTGCCCGGGGTTCTAGAC-3′
3′-GGAGGGGGGCGGAACGGGCCCCAAGATCTGCTAG-5′

PCR primers for IL-3, DB1, and β-actin were synthesized as follows; numbers correspond to the position relative to the translation initiation site.

IL-3 sense 5′-CTCCTGCCGATCCAAACATGAG-3′ (−17 to +5)
antisense 5′-AGAGGTTTCAGAAGTTCTGCTG-3′ (558 to 537)

DB1 sense 5′-TCTCAATCGACACAAGCTCT-3′ (527 to 546)
antisense 5′-AGATGTTACATGATACCTTG-3′ (837 to 818)

β-actin sense 5′-GAGAAGAGCTATGAGCTGCCT-3′ (709 to 729)
antisense 5′-TTCTGCATCCTGTCAGCAATG-3′ (944 to 924)

pUC00CAT was a kind gift from Kentaro Semba (IMSUT). This plasmid contains a simian virus 40 polyadenylation signal at the 5′ side of the multicloning site in order to reduce the background transcription level originating nonspecifically from the vector backbone. pUC00Luc was provided by Hyun Jun Lee (IMSUT). The chloramphenicol acetyltransferase (CAT) gene of pUC00CAT is replaced with the luciferase gene in this plasmid (18).

IL-3 deletion constructs linked to the firefly luciferase reporter gene were constructed by inserting the IL-3 promoter region into the multicloning site of pUC00Luc. IL-3 NheL, del 14, del 15, and del 17 contain regions from positions −650, −86, −56, and −25 to +47, respectively.

A mammalian expression vector, pME18S, was provided by Kazuo Maruyama (IMSUT). The longest inserts of EGR1, EGR2, and DB1 cDNAs were excised from the λgt11 vector by digestion with *Eco*RI and *Not*I and then were cloned into a 3.0-kb *Eco*RI-*Not*I fragment of pME18S to yield pME18S EGR1, pME18S EGR2, and pME18S DB1, respectively.

Fusion protein expression and purification. The entire coding region and the zinc finger portion of DB1 corresponding to positions −2 to 1556 and 499 to 956 relative to the translation initiation site were amplified by PCR and cloned into the *Bam*HI site of pGEX-3X and the *Eco*RI site of pGEX1, to produce pGEX-DB1-full and pGEX DB1-0.5, respectively. The plasmids were introduced into *Escherichia coli* AD202. The fusion proteins were induced as previously described (2) and purified by glutathione-Sepharose 4B, under conditions specified by the supplier (Pharmacia).

EMSA. Nuclear extract preparation and EMSA were done as described previously (25) but with minor modifications. The amount of poly(dI-dC) was changed to 0.5 μg per reaction mixture in this series of experiments. Absorption with antibody was performed by incubating the nuclear extract with the antibody for 3 h on ice and then by adding the binding buffer, poly(dI-dC), and a labeled oligonucleotide probe, CT/GC-wild. For competition experiments, each unlabeled oligonucleotide harboring a mutation (CT/GC-mut1 to -mut5) was added to the reaction mixture prior to the addition of the labeled probe.

Northern (RNA) blotting. mRNA purification, denaturing with glyoxal, electrophoresis, blotting, and hybridization were done as previously described (34). The blots were subjected to high-stringency washing. Multiple Tissue Northern blot was purchased from Clontech. Probe cDNA was labeled by random priming (34).

Reverse transcriptase-dependent PCR (RT-PCR). Total RNA was prepared from various cells, and 1 μg of total RNA or 0.1 μg of poly(A)$^+$ RNA was reverse transcribed with $(dT)_{12-18}$ as a primer, as previously described (34). Of the reverse-transcribed product, 1/20 was used for PCRs. One unit of *Taq* DNA polymerase (Kurashikibouseki) was used for each reaction mixture. PCR was done for 50, 30, and 28 cycles for DB1, IL-3, and β-actin, respectively (denatured at 96°C for 1 min, annealed at 55°C for 2 min, and extended at 72°C for 3 min).

Antibody. Antibodies to DB1 and EGR1 were raised against synthetic oligopeptides of deduced amino acid sequence (ETLKDAIGIKKEKPKTSFVC [see Fig. 2A] and KAFATQS GSQDLKALNTSY, respectively) linked to ovalbumin. The IgG fraction was purified from whole preimmune and immune sera with a protein A column (Pierce ImmunoPure IgG purification kit) and dialyzed against Dulbecco's phosphate-buffered saline (PBS) containing 10% glycerol.

In vitro transcription and translation. Whole cDNA of DB1 was cloned into the *Eco*RI-*Not*I fragment of pBluescript II SK− to produce pBSDB1. One microgram of pBSDB1 was transcribed and translated in vitro with a TNT T7 Coupled Reticulocyte Lysate System in a total reaction mixture volume of 25 μl. Two microliters was analyzed by electrophoresis in a sodium dodecyl sulfate (SDS)–10% polyacrylamide gel, dried, and autoradiographed.

Cells. The human T-cell leukemia line Jurkat was maintained in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM glutamine, 50 μM 2-mercaptoethanol, 50 U of penicillin per ml, and 50 μg of streptomycin per ml under 5% CO_2. Jurkat cells growing in the logarithmic phase were stimulated with PMA (Calbiochem, to a final concentration of 20 ng/ml) and A23187 (Calbiochem, to a final concentration of 0.5 μM) for 3 h to prepare the nuclear extract. For mRNA preparation, Jurkat cells were stimulated for the various periods given in the figures.

Transfection. Transfection and stimulation of Jurkat cells were done as described previously (15). To examine the effect of DNA-binding proteins on the IL-3 promoter-reporter constructs, 2.5 μg of reporter plasmids, 2.5 μg of cDNA incorporated into pME18S, and 1.25 μg of pcDSRαpX were used for transfection (23). pME18S vector was used to adjust the total amount of DNA transfected when necessary. Cells were harvested for the luciferase assay according to the manufacturer's instructions (Luciferase Assay System; Promega). Twenty microliters of cell extracts was used for one assay. The protein concentration of the cell extracts was measured with the bicinchoninic acid protein assay reagent (Pierce) with bovine serum albumin as a standard. Light units were calculated by using the following formula: (observed light units − background light units)/(micrograms of protein × seconds measured). Each transfection was repeated on at least three

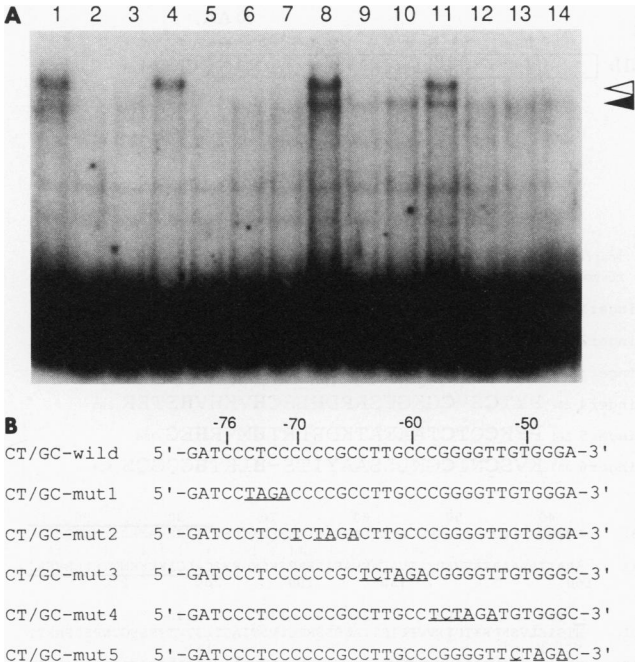

FIG. 1. EMSA of the human IL-3 CT/GC-rich element with Jurkat cell nuclear extract. (A) A radiolabeled CT/GC-rich region synthetic oligonucleotide was incubated with 4 μg of unstimulated (lanes 1 to 7) and stimulated (lanes 8 to 14) Jurkat nuclear extracts in the presence and absence of a 50-fold molar excess of competitors, and the resulting complexes were resolved in a nondenaturing 3.5% polyacrylamide gel. Lanes 1 and 8, without competitors; lanes 2 and 9, CT/GC-wild; lanes 3 and 10, CT/GC-mut1; lanes 4 and 11, CT/GC-mut2; lanes 5 and 12, CT/GC-mut3; lanes 6 and 13, CT/GC-mut4; lanes 7 and 14, CT/GC-mut5. Positions of the constitutively (open arrowhead) and the inducibly (closed arrowhead) appearing bands are indicated. (B) Sequence of the oligonucleotides used, with the mutated nucleotides underlined. Numbers given are relative to the transcription start site.

separate days, and representative data (see Fig. 8) or the average of data normalized to the value of Tax-cotransfected IL-3 NheL (see Fig. 9) is shown as the relative light unit.

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper will appear in the GSDB, DDBJ, EMBL, and NCBI nucleotide sequence databases under accession number D28118.

RESULTS

The IL-3 CT/GC-rich region forms a specific DNA-protein complex in the Jurkat cell nuclear extract. We previously reported that the CT/GC-rich region of the IL-3 gene is required for basal transcriptional activity and for the responsiveness to Tax. We earlier obtained evidence for specific protein complexes binding to this region (25). Since the binding of these complexes correlated well with the in vitro transcription activity (25), we characterized the protein(s) in these bands. When EMSA was performed with unstimulated and stimulated Jurkat cell nuclear extract and the CT/GC-rich region probe, one band which appears constitutively (Fig. 1A, lanes 1 and 8, open arrowhead) and the other which appears in a stimulation-dependent manner (lane 8, filled arrowhead) were observed. Both bands were specifically inhibited by a 50-fold excess of a cold probe, thereby indicating that these are sequence-specific complexes (lanes 2 and 9). Faint faster-migrating bands were not inhibited by the competitor and hence are due to nonspecific binding of the protein(s) included in the nuclear extract. A diffuse band with a slightly higher mobility than the inducible band appeared when we used an unstimulated extract (Fig. 1A, lane 1); however, we think that it is due to a nonspecific binding since it was not seen in other condition (see Fig. 7, lane 1) and was inhibited by all the competitors employed (Fig. 1A, lanes 2 to 7). The recognition sequence of the two specific bands was examined by competition with mutated oligonucleotides (Fig. 1B). The more slowly migrating band (Fig. 1A, open arrowhead) disappeared upon the addition of a 50-fold excess of mutated oligonucleotides, except CT/GC-mut2 (lanes 4 and 11). The stimulation-dependent, faster-migrating band was inhibited by CT/GC-mut3 to -mut5, and to a lesser extent with CT/GC-mut1. Thus, positions between −70 and −65 appear to be the major interacting region of both protein complexes, and −74 to −71 seems to be the minor interacting region of that with a higher mobility.

Cloning of cDNAs encoding the CT/GC-rich region binding factors reveals zinc finger proteins: EGR1, EGR2, and a novel protein, DB1. We screened a cDNA library of PMA- and A23187-stimulated Jurkat cells by Southwestern methods to search for a protein which specifically binds to the CT/GC-rich region. A probe which has three tandem repeats of the sequence (CCTCCCCCCGCCTTGC) was used. Of 300,000 independent plaques of oligo(dT)-primed λgt11 expression library, several distinct positive clones were isolated. Sequencing and homology searches revealed three clones to be identical to EGR1 (37) and one to be identical to EGR2 (13). Among the positive clones, two contained the same novel sequence not found in the GenBank and EMBL databases. This clone, DB1, gave the strongest signal and also bound to an IL-3 upstream monomer probe covering −100 to −39 (data not shown). We rescreened 900,000 plaques and obtained two containing DB1 cDNA. All the clones had approximately the same 5′ ends and various lengths of 3′ untranslated regions; they are classified into two types, S (short 3′ region) and L (long 3′ region). The longest type S clone was 2,320 bp long (Fig. 2A), and the type L clone was approximately 3.6 kbp long. The type L clone contained an additional ~1.3 kbp at the 3′ end of the type S clone. An in-frame ATG codon was found 50 bp downstream of the 5′ end. This ATG codon was in a reasonable context to serve as an initiation codon, according to Kozak (16, 17). S and L clones contained the same open reading frame that potentially encodes a protein of 516 amino acid residues and a predicted molecular mass of 56 kDa (Fig. 2A). In vitro transcription and translation revealed a polypeptide product with an apparent molecular mass of the expected size (Fig. 3). Upon analysis of the amino acid sequence, six potential zinc fingers of the Cys_2/His_2-type were found (Fig. 2A to C) (27). Five of the fingers conformed to the consensus $C\text{-}X_{2or4}\text{-}C\text{-}X_{12}\text{-}H\text{-}X_{3\text{–}5}\text{-}H$, while the sixth finger lacked one residue in the α helical region between the second Cys and the first His residues. The conserved hydrophobic residues, Pro, Phe or Tyr, Phe, and Leu at positions 1, 2, 11, and 17, respectively, as shown in Fig. 2C, were also conserved in fingers 1 to 5. Other characteristic features of the primary amino acid sequences are (i) a serine/threonine-rich composition throughout the protein, except in the zinc finger region, (ii) a glutamine stretch (Fig. 2A, broken underline), and (iii) periodically appearing proline residues in the C terminal 100 amino acids. Several direct repeats were present around these proline residues (PVT and PVTITSP).

Searches of the GenBank and EMBL databases by FASTA and TFASTA programs in December 1993 revealed several homologous sequences. The most closely related sequences

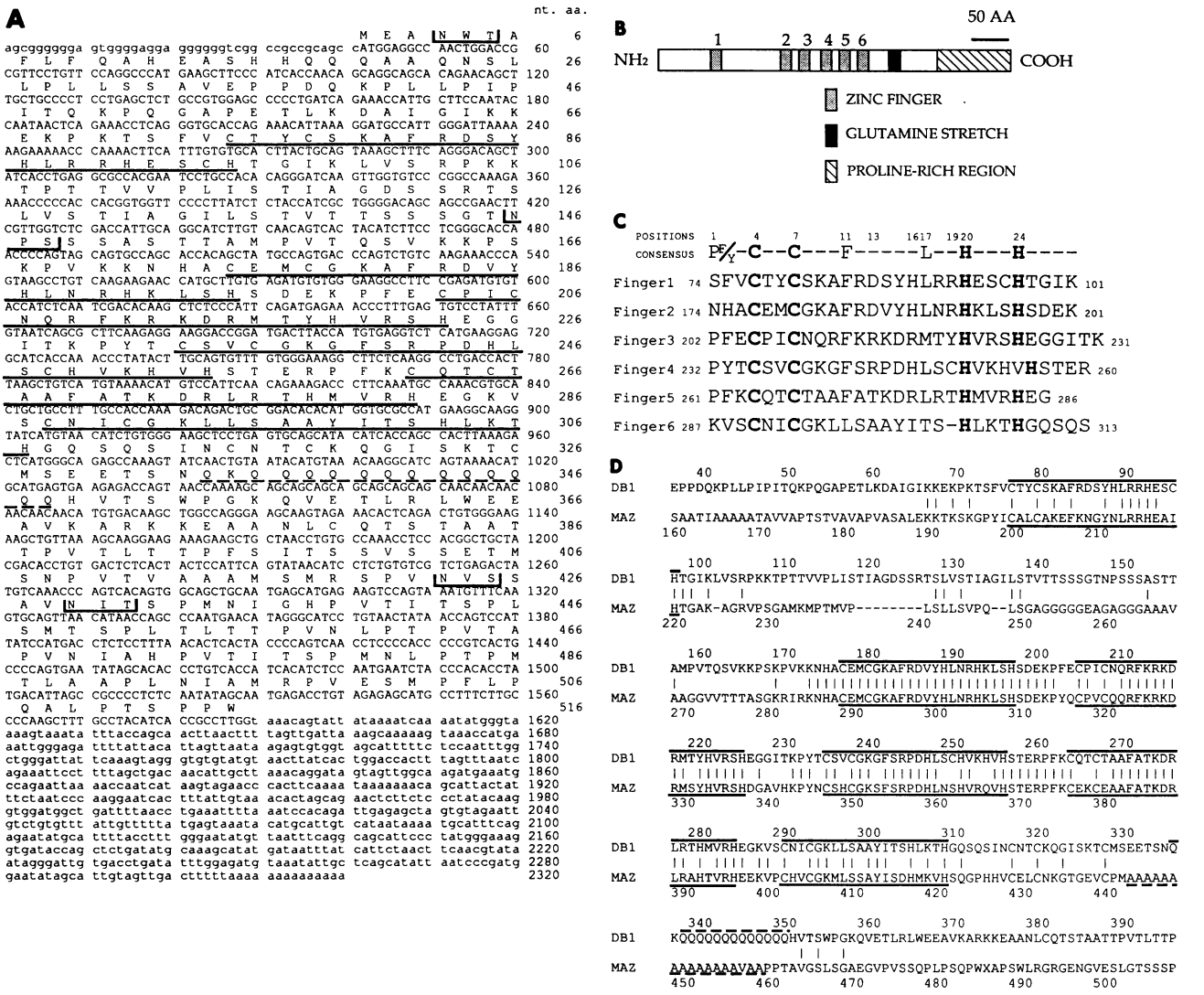

FIG. 2. (A) Nucleic acid and predicted amino acid sequence of cDNA encoding DB1. Zinc finger motifs are underlined, glutamine stretch is underlined by a broken line, and the putative N-glycosylation sites are indicated by brackets. nt., nucleotide numbers from the 5′ end of the cDNA; aa., amino acid numbers from the first in-frame Met. (B) Domains of the protein product of DB1, with scale bar indicating 50 amino acids (AA). (C) Alignments of the six zinc fingers with the consensus. The relative positions of the fingers are indicated by numbers. Numbers beside the amino acid sequences are the amino acid numbers from the first in-frame Met. (D) Comparison of amino acid sequences of DB1 and MAZ. Numbers denote the amino acid position from the first in-frame Met. The result is based on the homology search done with TFASTA (31). Identical amino acids are indicated by vertical bars. DB1 and MAZ share homology of 48.1% over 293 amino acids. The zinc finger regions, the glutamine stretch in DB1, and the alanine stretch in MAZ are indicated by horizontal bars and broken bars, respectively.

were human MAZ (ZF87) and its mouse homolog, Pur-1, which are 98.4% identical (3, 14, 33). MAZ (ZF87) was 66% identical to DB1 in 557 nucleotides overlap and 48% identical in 293 amino acids overlap (Fig. 2D). The homologous region started at 20 bp upstream of the second finger motif at the nucleotide level and at 10 amino acid residues N terminally to the first finger at the amino acid level. When the search was done at the nucleotide level, a region homologous to MAZ (ZF87) extended to the 3′ end of the sequence corresponding to the glutamine stretch of DB1, where the sequence was translated into an alanine stretch in MAZ (ZF87). There were no other proteins that showed a strong homology to DB1, except in the zinc finger region.

Northern blots and RT-PCR analysis revealed that DB1 mRNA has two species and is expressed ubiquitously in

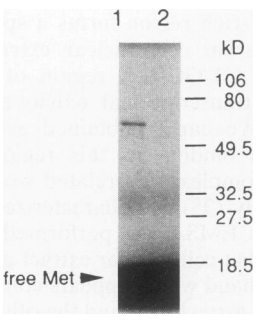

FIG. 3. SDS-polyacrylamide gel electrophoresis (PAGE) of in vitro-translated product of DB1. Lane 1, pBSDB1; lane 2, pBluescript II SK−. The apparent molecular masses estimated from prestained SDS-PAGE standards (Bio-Rad) are indicated.

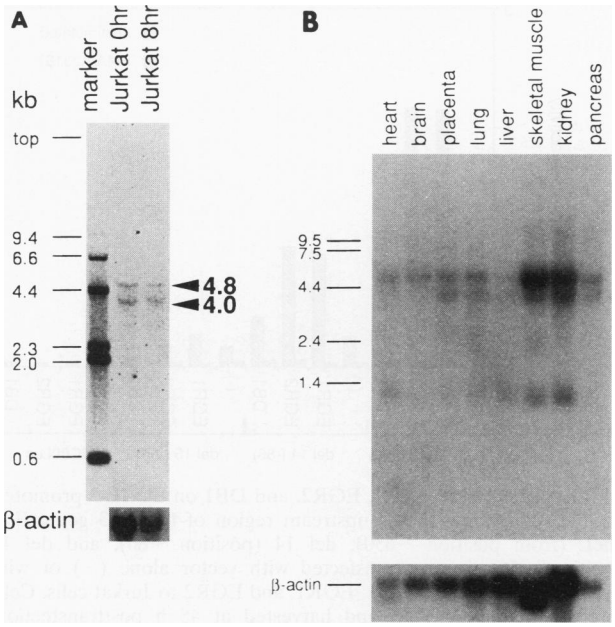

FIG. 4. Northern blot analysis of DB1 mRNA. (A) Five micrograms each of poly(A)$^+$ RNA from unstimulated and 8 h-stimulated Jurkat cells was electrophoresed, blotted onto nitrocellulose, and probed with ^{32}P-labeled DB1 cDNA. Positions of the molecular size markers of *Hin*dIII-digested λDNA are shown. Glyoxal-denatured RNA and DNA migrate at the same mobility in this condition. (B) Northern blot analysis of DB1 with various human tissue mRNAs. Two micrograms each of poly(A)$^+$ RNA from various tissues were blotted onto the filter.

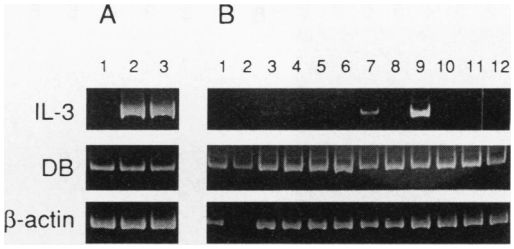

FIG. 5. RT-PCR analysis of DB1 and IL-3 with RNAs from Jurkat cells (A) and various cell lines and tissues (B). (A) The RNA samples are from Jurkat cells stimulated with PMA and A23187 for 0 h (unstimulated, lane 1), 8 h (lane 2), and 16 h (lane 3). (B) RNAs were from human peripheral blood monocytes treated with lipopolysaccharide for 18 h (lane 1); human peripheral blood monocytes treated with gamma interferon for 16 h (lane 2); WM-14, large granular lymphocytes/NK cells treated with PMA and A23187 for 15 h (lane 3); JR-2, a human B-cell lymphoma line, unstimulated (lane 4); TF-1, a human erythroleukemia cell line, unstimulated (lane 5); YT, large granular lymphocytes/NK cells, unstimulated (lane 6); 2F1, a human helper T-cell clone treated with concanavalin A for 3 h (lane 7); MLA144, a gibbon helper T-cell line, unstimulated (lane 8); Hy827, a human helper T-cell clone treated with PMA and A23187 for 8 h (lane 9); human placenta (lane 10); HL60, a human myelomonocytic leukemia line treated with PMA for 12 h (lane 11); and Jurkat, a human T-cell leukemia line, unstimulated (lane 12).

human tissues. Size of the DB1 mRNA transcript was determined by Northern blot analysis, as shown in Fig. 4A. Five micrograms of unstimulated and stimulated Jurkat poly(A)$^+$ mRNA were denatured by glyoxal and then were subjected to electrophoresis in phosphate buffer. DB1 was constitutively expressed as messages of 4.0 and 4.8 kb in length in Jurkat cells. The amount of DB1 mRNA seemed to be at a very low level, as deduced from the weak hybridization signal.

Tissue distribution of DB1 mRNA was tested by human multiple tissue Northern blot (Fig. 4B). The DB1 message was present in all the tissues tested, albeit in different amounts. The ratio of 4.8- to 4.0-kb transcript differed among tissues. The hybridization signal appearing at 1.2 kb may be due to nonspecific binding, since it did not appear in Fig. 4A and it is too small for the size of the DB1 cDNA.

mRNAs from various hemopoietic cells and placenta were tested for the presence of DB1 and IL-3 mRNA by RT-PCR (Fig. 5). Primers specific to IL-3, DB1, and β-actin were synthesized to give PCR products of 575, 311, and 236 bp, respectively. The amount of DB1 mRNA did not change upon stimulation of Jurkat cells with PMA and A23187, while the IL-3 message was absolutely inducible (Fig. 5A). The same samples were subjected to lower cycles of PCR to allow for a quantitative analysis, which gave the same result (data not shown). IL-3 mRNA was detected in PMA- and A23187-treated WM-14 (NK cell), concanavalin A-stimulated 2F1 (T cell), PMA- and A23187-treated Hy827 (T cell), and PMA- and A23187-treated Jurkat cells (T cell), but not in lipopolysaccharide- or gamma interferon-stimulated human peripheral blood monocytes, unstimulated JR-2 (B cell), unstimulated TF-1 (erythroleukemia cell line), unstimulated YT (NK cell), unstimulated MLA144 (gibbon T lymphoma), human placenta, PMA-treated HL60 (myelomonocytic leukemia), and unstimulated Jurkat cells (Fig. 5B). On the other hand, DB1 mRNA was detected in all the cells tested; hence, DB1 is widely expressed in human blood cells (Fig. 5B). There was no obvious correlation between expression of IL-3 and DB1 mRNA.

Recombinant DB1 expressed in *E. coli* binds to the CT/GC-rich region. To confirm the direct binding of DB1 protein to the CT/GC-rich region, DB1 protein was expressed in bacteria. Glutathione *S*-transferase fusion proteins batch purified with glutathione-Sepharose 4B were subjected to competition analysis in EMSA. Full-length DB1 protein expressed in bacteria showed the same binding specificity as the more slowly migrating band detected in Fig. 1A (Fig. 6A, open arrowhead). Smear bands with a higher mobility appearing at the bottom of the gel are due to degradation products of the fusion protein and were confirmed by Western blotting (immunoblotting). When only the zinc finger portion (fingers, 2 to 6) was expressed, it exhibited the same binding specificity (Fig. 6B), thereby indicating that finger 1 is not directly involved in the sequence-specific DNA-binding activity.

DB1 and EGR1 proteins are the components of specific protein complexes detected in EMSA. We then identified the bands observed in EMSA (Fig. 7). Polyclonal antibodies against synthetic peptides of DB1 and EGR1 were developed, and the IgG fraction was developed from sera, which we denote αDB1 and αEGR1, respectively. Preimmune serum was processed similarly, to yield control IgG. EMSA was done under the same condition as that of Fig. 1A. When the nuclear extract was preincubated with αDB1, the upper band was specifically absorbed and a supershifted band was generated (Fig. 7, lanes 3 and 7). The lower band disappeared by preincubation with αEGR1 (lane 8). Absorption was not observed with buffer alone (lanes 1 and 5) or with control IgG (lanes 2 and 6). This result indicates that the constitutively appearing band with a lower mobility includes DB1 protein while the stimulation-dependent band with higher mobility comprises EGR1 protein.

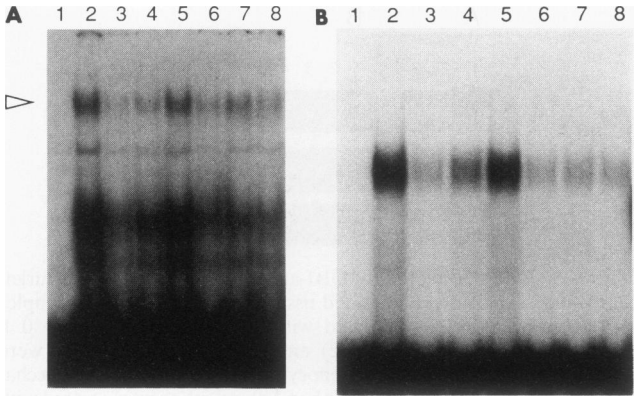

FIG. 6. DB1 protein expressed in bacteria binds specifically to the CT/GC-rich region. cDNA fragments corresponding to the whole coding region (A) and zinc finger motifs 2 to 6 (B) of DB1 were cloned into expression plasmids. Bacteria containing the pGEX-3X vector (A and B, lane 1), or the pGEX-DB1-full (A, lanes 2 to 8) and pGEX-DB1-0.5 (B, lanes 2 to 8) constructs were treated with 0.1 mM isopropyl-β-D-thiogalactopyranoside for 2 h and then lysed by sonication. The fusion proteins were purified and subjected to EMSA, as described in Materials and Methods. A 25-fold excess of each cold oligonucleotide was used for competition. Lanes 1 and 2, without competitors; lane 3, CT/GC-wild; lane 4, CT/GC-mut1; lane 5, CT/GC-mut2; lane 6, CT/GC-mut3; lane 7, CT/GC-mut4; lane 8, CT/GC-mut5. The position of intact fusion protein is indicated in panel A (open arrowhead).

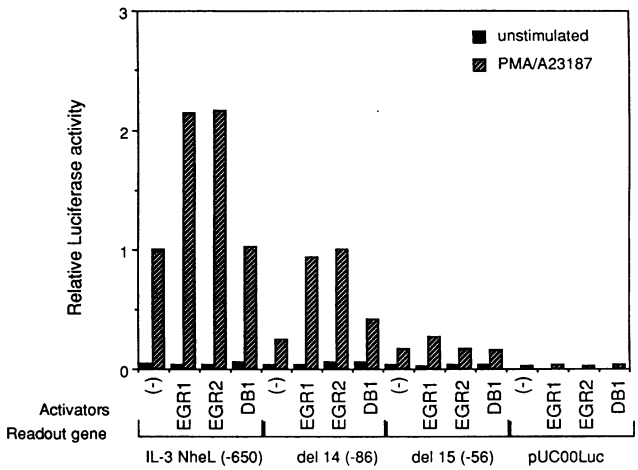

FIG. 8. Effects of EGR1, EGR2, and DB1 on the IL-3 promoter. Plasmids containing the 5′ upstream region of the IL-3 gene, IL-3 NheL (from position −650), del 14 (position −86), and del 15 (position −56) were cotransfected with vector alone (−) or with expression plasmids for DB1, EGR1, and EGR2 to Jurkat cells. Cells were stimulated at 40 h and harvested at 48 h posttransfection. Luciferase activity was measured as described in Materials and Methods.

EGR1 and EGR2 contribute to the PMA- and A23187-dependent transcription of IL-3 in Jurkat cells. To determine whether EGR1, EGR2, and DB1 have any effect on transcription of the IL-3 gene, cotransfection experiments of the expression plasmid of EGR1, EGR2, or DB1 with the IL-3 promoter-reporter constructs were carried out with Jurkat cells, a human T-cell leukemia cell line (Fig. 8). We constructed three reporter genes, IL-3 NheL, del 14, and del 15. Each contained a firefly luciferase gene linked to various regions of the human IL-3 promoter: from −650 to +47 (IL-3 NheL), from −86 to +47 (del 14), and from −56 to +47 (del 15). Plasmid IL-3 NheL contains a binding site for AP1, NF-IL3-A/ACT-1, NIP, and the CT/GC-rich region, all of which have been reported to be a regulatory sequence for IL-3

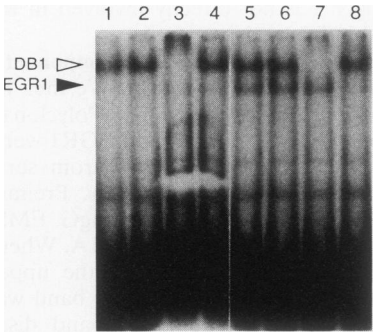

FIG. 7. Identification of the bands detected in EMSA. Unstimulated (lanes 1 to 4) and stimulated (lanes 5 to 8) Jurkat nuclear extracts were preincubated for 3 h on ice with PBS–10% glycerol (lanes 1 and 5), 5 μg of control IgG (lanes 2 and 6), αDB1 (lanes 3 and 7), and αEGR1 (lanes 4 and 8). Bands corresponding to those in Fig. 1A are labeled and indicated by open and closed arrowheads.

transcription. Plasmid del 14 contains the CT/GC-rich region, while del 15 lacks the 5′ half of this region which is the binding site for DB1 and is also a homologous sequence of the EGR recognition site (CGCCCCCGC) (4). All the constructs had little or no basal promoter activity when the cells were not stimulated. Stimulation by either PMA or A23187 alone did not induce transcription activity of the IL-3 promoter (data not shown). Stimulation by a combination of PMA and A23187 enhanced transcription from IL-3 NheL by 22-fold. del 14 and del 15 were activated by 7.6- and 6.1-fold over unstimulated levels, respectively. We regard this as nonspecific activation, since pUC00Luc was also activated by PMA and A23187 stimulation by eightfold. Transfection of expression vectors of EGR1, EGR2, or DB1 did not alter transcriptional activity of the reporter plasmids when the cells were not stimulated (Fig. 8). When the cells were stimulated, EGR1 and EGR2 augmented the effect of PMA and A23187 on IL-3 NheL by twofold. The enhancement by EGR1 and EGR2 was also observed in del 14, which includes the CT/GC-rich region, but not in del 15, which lacks the 5′ half of the CT/GC-rich region.

These results indicate that the enhancing activity of EGR1 and EGR2 depends on the CT/GC-rich region and requires stimulation of the cells with PMA and A23187. The enhanced activity of IL-3 NheL was higher than that of del 14, possibly owing to the presence of upstream PMA- and A23187-responsive elements such as AP1 and NF-IL3-A/ACT-1.

DB1 acts in synergy with Tax to stimulate transcription from the IL-3 promoter. The CT/GC-rich region in the IL-3 promoter was originally defined as a responsive element for Tax *trans* activation (25). To test whether EGR1, EGR2, or DB1 affects transcriptional activation by Tax, the expression plasmid for Tax was cotransfected with the DB1 expression plasmid and the reporter plasmids (Fig. 9).

Cotransfection of Tax expression vector activated transcription of IL-3 NheL, del 14, and del 15 (Fig. 9). It also exhibited a nonspecific transcription enhancement on del 17 (contains −25 to +47 of the promoter region) and pUC00Luc. The effect of Tax was prominent on plasmid del 14 and lower in

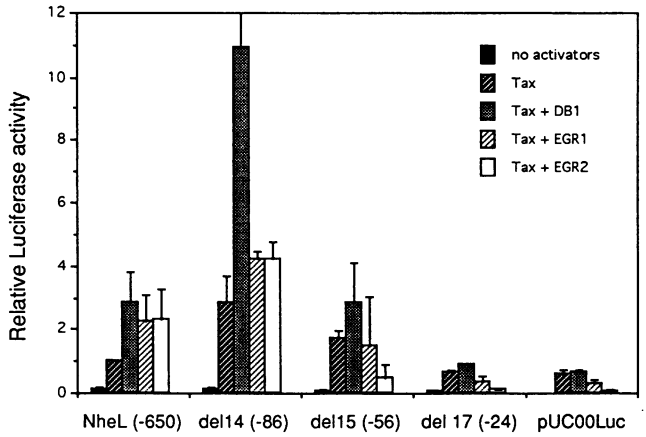

FIG. 9. Cotransfection effects of Tax with EGR1, EGR2, and DB1 on the IL-3 promoter. Tax and expression vectors of EGR1, EGR2, and DB1 were cotransfected with IL-3 reporter plasmids to Jurkat cells. Luciferase activity was measured as described in Materials and Methods.

longer promoter constructs, findings consistent with our previous results with the CAT reporter gene (25).

Cotransfection of DB1 expression plasmid with IL-3 NheL or del 14 augmented Tax-dependent transcription activity by approximately threefold (Fig. 9). The enhancing effect of DB1 was not observed with reporter plasmids del 15, del 17, and pUC00Luc (Fig. 9), and without Tax (Fig. 8); therefore, this activity probably depends on the CT/GC-rich region and Tax. Cotransfection of EGR1 and EGR2 expression vectors had no apparent effect on the Tax-dependent transcription activity of the reporter plasmids (Fig. 9).

DISCUSSION

The CT/GC-rich region of the IL-3 promoter sustains basal promoter activity and is a Tax response element (15, 25). This sequence is not found in other lymphokine gene promoters and may play a role to support basal transcriptional activity by interacting with DNA-binding proteins in an IL-3 gene-specific manner. In this report, we described the cloning of cDNAs encoding proteins that bind to this region.

Three cDNA clones were identified: EGR1, EGR2, and DB1, the latter being a novel one. Since the first in-frame ATG of DB1 was in a reasonable context to serve as an initiation codon, according to Kozak (16, 17), we assume that the isolated cDNA clones are nearly full length with regard to coding region. The DB1 mRNA detected in Jurkat cells was of two sizes, 4.0 and 4.8 kb (Fig. 4A), while the isolated cDNA clones were 2.3 and 3.6 kb long, respectively. This difference may be due to length of the poly(A) tail. However, it is also possible that the upstream transcription initiation site is several hundred bases upstream, and the present cDNA still lacks the 5′ sequence. We examined the length of the 5′ upstream region, by primer extension analysis and by the anchored PCR method, with two independent primers, but we detected no distinct cDNA end. This may be due to the high GC content (approximately 80%) of the mRNA spanning for 60 bases upstream of the first ATG codon, which is known to be difficult for reverse transcriptase to completely transcribe. It should be noted that the GC-rich sequence is characteristic of the 5′ flanking region of the coding region. The DB1 mRNA was expressed in all the tissues and cell lines tested, and the amount did not change upon T-cell stimulation. This agrees with our observation that the DB1-containing band detected in EMSA remained unchanged after T-cell activation (Fig. 1A and 7).

The apparent size of the DB1 protein expressed in vitro was almost the same as the calculated molecular mass which is 56 kDa. However, DB1 protein expressed in COS7 cells had an apparent size of 80 kDa (data not shown). This discrepancy may be due to posttranslational modifications, including glycosylation, since the predicted sequence contains four possible N-linked glycosylation sites (Fig. 2A).

The structures of Egr-1, GL1, and Tramtrack, transcription factors having Cys_2/His_2-type zinc finger motifs, have been well characterized by X-ray crystallography and nuclear magnetic resonance spectroscopy. It was demonstrated that each zinc finger motif forms a modular structure to bind DNA (8, 24, 29, 30). DB1 protein having six Cys_2/His_2-type zinc finger motifs was predicted to bind to DNA. Indeed, the DB1 protein expressed in bacteria bound to the CT/GC-rich region probe with the same specificity as the upper band observed when Jurkat cell nuclear extract was used (Fig. 1A), and zinc fingers 2 to 6 were sufficient to confer this specificity (Fig. 6A and B). The protein complex which was shown to include EGR1 (Fig. 1A and 7) appeared upon T-cell activation and showed the same sequence requirement as in the previous report (4). Alignment of the zinc finger motifs of DB1 protein with the consensus sequence (7) revealed that hydrophobic residues at positions 1, 2, 11, and 17 which are known to form hydrophobic interactions to stabilize the structure of the zif fingers (29) are well conserved from fingers 1 to 5 (Fig. 2C). The sixth finger lacked all these conserved hydrophobic residues and also lacked one amino acid residue in the α-helical region; hence, it seems unlikely that the sixth finger forms a functional DNA-binding motif.

Although there is no simple general code describing protein-DNA interaction of various zinc finger proteins, the overall structures of the zinc fingers are generally conserved, and there seems to be a correlation between the position of the amino acid residue in the zinc finger and the recognition site of the DNA sequence (8, 30). By comparing the amino acid sequences of the DB1 and zif268 (Egr-1) zinc fingers, we found several residues involved in protein-DNA interaction in the zif fingers to be identical to that placed at the same position in the DB1 fingers. In the zif complex, the residue immediately preceding the α-helical region (position 13 in Fig. 2C) is Arg and contacts the third base on the primary strand of the subsite (5′- -G). The third residue in the α-helix (position 16, His) can contact the second base on the primary strand (5′-G-), and the sixth residue (position 19, Arg) in the α-helix can contact the first base (5′ G- -) of the subsite. In the case of DB1, position 13 is Arg in fingers 3 and 4. Position 16 is His in fingers 1, 2, and 4, and position 19 is Arg in fingers 1 and 2. Thus, it is likely that DB1 contacts a sequence similar to the recognition site of Egr-1. Fingers 2 to 5 of DB1 also show strong homology to MAZ (ZF8)7 zinc fingers (Fig. 2D). The identity is consistent with the fact that DB1, EGR1, and MAZ (ZF87) bind to homologous sequences. Conversely, it is also possible that DB1 binds to the ME1a1 or ME1a2 element of the *myc* promoter which is the recognition site of MAZ (ZF87) (3) or to the recognition sequences of EGR family proteins.

Other characteristic features of DB1 are Ser/Thr-rich composition outside the zinc finger portion, existence of a glutamine stretch, and periodically appearing Pro residues. At present, there is no evidence for the involvement of the Ser/Thr-rich region in transcription; however, serving as a potential site for glycosylation, these Ser/Thr-rich regions

might play an important role in regulating some aspect of DB1 function. Glutamine stretch and proline-rich regions are found in several transcription factors and activate (Sp1 [5], AP-2 [41], OTF-2 [11], and CTF/NF-I [22]) or repress (WT1 [20]) transcription. Thus, it is likely that these two domains are regulatory regions within this protein. Interestingly, the sequence of MAZ (ZF87) corresponding to the glutamine stretch of DB1 is an alanine stretch, which arises by shifting the reading frame (Fig. 2D). The alanine stretch is also found in several transcription factors (33). Considering the fact that DB1 and MAZ (ZF87) have homology only through the zinc finger portion and that the frame is shifted in the glutamine stretch, we speculate that these genes evolved from a common ancestor, conserving the zinc finger portion.

In this study, we used a series of deletion IL-3 reporter plasmids and the luciferase reporter gene system. These reporter plasmids responded well to signals activated by PMA and A23187 (Fig. 8), a finding in contrast to previous observations (25). This may be due to differences in sensitivity between the CAT and luciferase reporter systems. Requirement for the CT/GC-rich region by Tax was reproduced by the luciferase constructs (Fig. 9). The response to Tax was weaker with the plasmid containing longer upstream sequences, which is consistent with our previous findings and suggests that the upstream sequence may include negative DNA elements to Tax activation.

Cotransfection of the expression vectors of EGR1 and EGR2 cDNA with IL-3 promoter-luciferase plasmid enhanced IL-3 transcription in the presence of PMA and A23187 stimulation. Since EGR1 bound to the CT/GC-rich region probe (Fig. 7), we assume that EGR1 takes part in the inducible expression of the IL-3 gene. A protein complex that includes EGR2 was not detected in our EMSA system, using the anti-EGR2 antibody. Since only two major bands detected in EMSA contained DB1 and EGR1, it seems that EGR2 is not a major member binding to the CT/GC-rich region. Therefore, although we did observe activation of the IL-3 promoter by EGR2 in a transient-transfection system, we cannot conclude that EGR2 is involved in inducible expression of the IL-3 gene.

Although EGR1 and EGR2 activated transcriptional activity of the native IL-3 promoter, when we used three tandem copies of the CT/GC-rich region hooked to a thymidine kinase minimal promoter-luciferase gene as a template [$(CT/GC)_3$-TK-Luc], no apparent effect was observed (data not shown). This indicates that the CT/GC-rich region does not by itself have the ability to mediate T-cell activation signals transmitted through EGR1 and EGR2. Rather, the CT/GC-rich region appears to positively modulate the transcription level of the IL-3 gene in the context of the native promoter. Flanking regions of the CT/GC-rich region (-86 to -77 and/or -45 to $+47$), which are present in the native promoter but not in the tandem constructs, may be one of the sequences that confer the missing function.

Cotransfection of DB1 expression vector augmented the Tax effect on IL-3 NheL and del 14 (Fig. 9), and EGR1 and EGR2 had no effect on these reporter plasmids. Several sequences including the 21-bp enhancer of the human T-cell leukemia virus type I long terminal repeat (10), NF-κB binding site (19), and SRE (1) have been reported to be Tax-responsive elements which do not share homology with the CT/GC-rich region. Thus, the CT/GC-rich region seems to be a novel DNA-binding motif that mediates the Tax effect. There is no sequence homology among Tax-responsive elements, and no direct binding of Tax to those sequences has been demonstrated. Instead, an association of DNA-binding proteins with Tax has been reported (9, 12, 38, 40). Although it still remains to be determined whether DB1 directly interacts with Tax to activate the transcriptional machinery, our result suggests that DB1 does mediate the Tax effect in *trans*-activating the IL-3 gene.

DB1 is a ubiquitous and constitutively expressed protein; it binds to the CT/GC-rich region, and the mutation of its recognition sequence decreases the basal transcription activity (25). Thus, DB1 is a candidate protein that supports the basal transcriptional activity of the IL-3 promoter, in Jurkat cells. We also suggest that DB1 serves as a Tax-interacting molecule in cells expressing Tax. To gain support for the role of DB1 as well as EGR1 and EGR2 in IL-3 transcription, the inhibition of protein expression by antisense nucleotides or depleting the proteins from the nuclear extract in in vitro transcription assays will need to be done.

ACKNOWLEDGMENTS

We thank Masa-aki Muramatsu, Fernando Bazan, Akio Tsuboi, Mitsuo Watanabe, Hyun Jun Lee, Lisako Tsuruta, Yasushi Nakagawa, and Mariko Ohara for critical reading of the manuscript and for helpful discussions. We are also grateful to the staff at the Laboratory of Molecular Genetics, IMSUT, for synthesizing the oligonucleotides and to Patricia Meyerson at DNAX research institute for technical assistance and DNA sequencing. Computational time was provided by the Human Genome Center, IMSUT.

This work was supported by a Grant-in-Aid for Scientific Research (B) (T.Y.), a Grant-in-Aid for Cancer Research (K.-I.A.), the Ministry of Education, Science and Culture of Japan, and JSPS Fellowships for Japanese Junior Scientists (N.K.-N.). The DNAX Research Institute of Molecular and Cellular Biology is supported by the Schering-Plough Corporation.

REFERENCES

1. **Alexandre, C., and B. Verrier.** 1991. Four regulatory elements in the human c-fos promoter mediate transactivation by HTLV-I Tax protein. Oncogene **6**:543–551.
2. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1989. Current protocols in molecular biology. Greene Publishing Associates/Wiley-Interscience, New York.
3. **Bossone, S. A., C. Asselin, A. J. Patel, and K. B. Marcu.** 1992. MAZ, a zinc finger protein, binds to c-MYC and C2 gene sequences regulating transcriptional initiation and termination. Proc. Natl. Acad. Sci. USA **89**:7452–7456.
4. **Cao, X., R. A. Koski, A. Gashler, M. McKiernan, C. F. Morris, R. Gaffney, R. V. Hay, and V. P. Sukhatme.** 1990. Identification and characterization of the Egr-1 gene product, a DNA-binding zinc finger protein induced by differentiation and growth signals. Mol. Cell. Biol. **10**:1931–1939.
5. **Courey, A. J., and R. Tjian.** 1988. Analysis of Sp1 in vivo reveals multiple transcriptional domains, including a novel glutamine-rich activation motif. Cell **55**:887–898.
6. **Davies, K., E. C. TePas, D. G. Nathan, and B. Mathey-Prevot.** 1993. Interleukin-3 expression by activated T cells involves an inducible, T-cell-specific factor and an octamer binding protein. Blood **81**:928–934.
7. **Evans, R. M., and S. M. Hollenberg.** 1988. Zinc fingers: gilt by association. Cell **52**:1–3.
8. **Fairall, L., J. W. R. Schwabe, L. Chapman, J. T. Finch, and D. Rhodes.** 1993. The crystal structure of a two zinc-finger peptide reveals an extension to the rules for zinc-finger/DNA recognition. Nature (London) **366**:483–487.
9. **Fujii, M., H. Tsuchiya, T. Chujoh, T. Akizawa, and M. Seiki.** 1992. Interaction of HTLV-I Tax 1 with $p67^{SRF}$ causes the aberrant induction of cellular immediate early genes through CArG boxes. Genes Dev. **6**:2066–2076.
10. **Fujisawa, J., M. Toita, and M. Yoshida.** 1989. A unique enhancer element for the trans activator ($p40^{tax}$) of human T-cell leukemia virus type I that is distinct from cyclic AMP- and 12-*O*-tetradecanoylphorbol-13-acetate-responsive elements. J. Virol. **63**:3234–3239.
11. **Gerster, T., C.-G. Balmaceda, and R. G. Roeder.** 1990. The cell

type-specific octamer transcription factor OTF-2 has two domains required for the activation of transcription. EMBO J. **9**:1635–1643.

12. **Hirai, H., J. Fujisawa, T. Suzuki, K. Ueda, M. Muramatsu, A. Tsuboi, N. Arai, and M. Yoshida.** 1992. Transcriptional activator Tax of HTLV-1 binds to the NF-κB precursor p105. Oncogene **7**:1737–1742.
13. **Joseph, L. J., B. M. Le, G. J. Jamieson, S. Acharya, T. B. Shows, J. D. Rowley, and V. P. Sukhatme.** 1988. Molecular cloning, sequencing, and mapping of EGR2, a human early growth response gene encoding a protein with "zinc-binding finger" structure. Proc. Natl. Acad. Sci. USA **85**:7164–7168. (Erratum **86**:515, 1989.)
14. **Kennedy, G. C., and W. J. Rutter.** 1992. Pur-1, a zinc-finger protein that binds to purine-rich sequences, transactivates an insulin promoter in heterologous cells. Proc. Natl. Acad. Sci. USA **89**:11498–11502.
15. **Koyano-Nakagawa, N., J. Nishida, N. Arai, K.-i. Arai, and T. Yokota.** 1993. Reconstitution of the functional GM-CSF promoter: evidence for distinct activation mechanisms that mediate the response to phorbol ester/calcium signals. Int. Immunol. **5**:345–342.
16. **Kozak, M.** 1984. Compilation and analysis of sequences upstream from the translational start site in eukaryotic mRNAs. Nucleic Acids Res. **12**:857–872.
17. **Kozak, M.** 1987. At least six nucleotides preceding the AUG initiator codon enhance translation in mammalian cells. J. Mol. Biol. **196**:947–950.
18. **Lee, H. J., J. Nishida, N. Koyano-Nakagawa, Y. Naito, K. Arai, and T. Yokota.** 1993. cAMP activates the IL-5 promoter synergistically with phorbol ester through the signalling pathway involving protein kinase A in mouse thymoma line EL-4. J. Immunol. **151**:6135–6142.
19. **Leung, K., and G. J. Nabel.** 1988. HTLV-1 transactivator induces interleukin-2 receptor expression through an NF-κB-like factor. Nature (London) **333**:776–778.
20. **Madden, S. L., D. M. Cook, J. F. Morris, A. Gashler, V. P. Sukhatme, and F. J. Rauscher III.** 1991. Transcriptional repression mediated by the WT1 Wilms tumor gene product. Science **253**:1550–1553.
21. **Mathey-Prevot, B., N. C. Andrews, H. S. Murphy, S. G. Kreissman, and D. G. Nathan.** 1990. Positive and negative elements regulate human interleukin 3 expression. Proc. Natl. Acad. Sci. USA **87**:5046–5050.
22. **Mermod, N., E. A. O'Neill, T. J. Kelly, and R. Tjian.** 1989. The Proline-rich transcriptional activator of CTF/NF-I is distinct from the replication and DNA binding domain. Cell **58**:741–753.
23. **Miyatake, S., M. Seiki, M. Yoshida, and K. Arai.** 1988. T-cell activation signals and human T-cell leukemia virus type I-encoded $p40^x$ protein activate the mouse granulocyte-macrophage colony-stimulating factor gene through a common DNA element. Mol. Cell. Biol. **8**:5581–5587.
24. **Nardelli, J., T. J. Gibson, C. Vesque, and P. Charnay.** 1991. Base sequence discrimination by zinc-finger DNA-binding domains. Nature (London) **349**:175–178.
25. **Nishida, J., M. Yoshida, K.-i. Arai, and T. Yokota.** 1991. Definition of a GC-rich motif as regulatory sequence of the human IL-3 gene: coordinate regulation of the IL-3 gene by CLE2/GC box of the GM-CSF gene in T cell activation. Int. Immunol. **3**:245–254.
26. **Otsuka, T., A. Miyajima, N. Brown, K. Otsu, J. Abrams, S. Saeland, C. Caux, R. de Waal Malefijt, J. de Vries, P. Meyerson, K. Yokota, L. Gemmell, D. Rennick, F. Lee, K. Arai, and T. Yokota.** 1988. Isolation and characterization of an expressible cDNA encoding human IL-3. Induction of IL-3 mRNA in human T cell clones. J. Immunol. **140**:2288–2295.
27. **Pabo, C. O., and R. T. Sauer.** 1992. Transcription factors: structural families and principles of DNA recognition. Annu. Rev. Biochem. **61**:1053–1095.
28. **Park, J.-H., K. Kaushansky, and L. Levitt.** 1993. Transcriptional regulation of interleukin 3 (IL3) in primary human T lymphocytes. Role of AP-1- and octamer-binding proteins in control of IL3 gene expression. J. Biol. Chem. **268**:6299–6308.
29. **Pavletich, N. P., and C. O. Pabo.** 1991. Zinc finger-DNA recognition: crystal structure of a Zif268-DNA complex at 2.1Å. Science **252**:809–817.
30. **Pavletich, N. P., and C. O. Pabo.** 1993. Crystal structure of a five-finger GLI-DNA complex: new perspectives on zinc fingers. Science **261**:1701–1707.
31. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85**:2444–2448.
32. **Plaut, M., J. H. Pierce, C. J. Watson, H. J. Hanley, R. P. Nordan, and W. E. Paul.** 1989. Mast cell lines produce lymphokines in response to cross-linkage of Fc epsilon RI or to calcium ionophores. Nature (London) **339**:64–67.
33. **Pyrc, J. J., K. H. Moberg, and D. J. Hall.** 1992. Isolation of a novel cDNA encoding a zinc-finger protein that binds to two sites within the c-myc promoter. Biochemistry **31**:4102–4110.
34. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
35. **Shoemaker, S. G., R. Hromas, and K. Kaushansky.** 1990. Transcriptional regulation of interleukin 3 gene expression in T lymphocytes. Proc. Natl. Acad. Sci. USA **87**:9650–9654.
36. **Sonoda, Y., Y.-C. Yang, G. G. Wong, S. C. Clark, and M. Ogawa.** 1988. Analysis in serum-free culture of the targets of recombinant human hemopoietic growth factors: interleukin 3 and granulocyte/macrophage-colony-stimulating factor are specific for early developmental stages. Proc. Natl. Acad. Sci. USA **85**:4360–4364.
37. **Sukhatme, V. P., X. Cao, L. C. Chang, C. H. Tsai-Morris, D. Stamenkovich, P. C. P. Ferreira, D. R. Cohen, S. A. Edwards, T. B. Shows, T. Curran, M. M. Le Beau, and E. D. Adamson.** 1988. A zinc finger-encoding gene coregulated with c-fos during growth and differentiation, and after cellular depolarization. Cell **53**:37–43.
38. **Suzuki, T., J. Fujisawa, M. Toita, and M. Yoshida.** 1993. The trans-activator Tax of human T-cell leukemia virus type 1 (HTLV-1) interacts with cAMP-responsive element (CRE) binding and CRE modulator proteins that bind to the 21-base-pair enhancer of HTLV-1. Proc. Natl. Acad. Sci. USA **90**:610–614.
39. **Ullman, K. S., J. P. Northrop, C. L. Verweij, and G. R. Crabtree.** 1990. Transmission of signals from the T lymphocyte antigen receptor to the genes responsible for cell proliferation and immune function: the missing link. Annu. Rev. Immunol. **8**:421–452.
40. **Wagner, S., and M. R. Green.** 1993. HTLV-I Tax protein stimulation of DNA binding of bZIP proteins by enhancing dimerization. Science **262**:395–399.
41. **Williams, T., and R. Tjian.** 1991. Analysis of the DNA-binding and activation properties of the human transcription factor AP-2. Genes Dev. **5**:670–682.
42. **Yang, Y.-C., A. B. Ciarletta, P. A. Temple, M. P. Chung, S. Kovacic, J. S. Witek-Giannotti, A. C. Leary, R. Kriz, R. E. Donahue, G. G. Wong, and S. C. Clark.** 1986. Human IL-3 (multi-CSF): identification by expression cloning of a novel hematopoietic growth factor related to murine IL-3. Cell **47**:3–10.